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PROMOTION OF ANIMAL PRODUCTS AS OPPORTUNITY FOR FURTHER DEVELOPMENT OF BREEDING

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Invited paper

Abstract: In order to ensure safety of meat and meat products as well as necessary repeatability of products quality throughout the food chain, raw materials producers are required to ensure appropriate condition for production (both crops and breeding). It obligates to implementation and application systems such as: Good Agriculture Practise (GAP), Good Manufacturing Practice (GMP), Good Higiene Practice (GHP), and for intermediary companies participating in raw materials trade mandatory implementation of GHP and GMP system as well as HACCP (Hazard Analysis and Critical Control Point), QACP (Quality Assurance Control Points), ISO 9001, ISO 22000. In 1987 was established ISO9000 series of standards by International Organization of Standardization for concerning quality management and quality assurance. In this system quality control is continuous and take place at every step from design through production to waste disposal. Application of management systems requires reconstruct and expansion of information flow's channel between processing (meat processing plant) and raw materials suppliers (breeders of animal of slaugters) regarding rules of preparation and implication of feeds and drugs. Ensuring repeatability quality of producing meat products demand managing by meat processing plant or appointed to this institution, breeders audit and monitoring of livestock and crops. For proper functioning of quality management systems is necessary ensure highest quality in whole chain food, ie from farm to table. Hazard Analysis and Critical Control Point (HACCP) is quality management system, for food production process, which was imtroduce by UE Directive No. 92/46 and is valid in Poland mainly so that the producer can become a reliable supplier of food on the EU market. Adjustment of Polish law to European Union regulations require many activities including the dissemination of the principles of GMP and implementation of HACCP system in food processing (according to Directive EU 93/94/ECC in foodstuffs hygiene). HACCP system is considered as most effective and most efficient tool in ensure

high standard of hygiene condition of production and food processing. Generally, HACCAP system can be described as procedures designed to identify the health hazards of food and the risk of their occurrence during all stages of food production and distribution. This is system, which controls and protects risks relevant to consumers safety and their health protect protection. HACCP protects consumer interests giving him assurance of safety and high health quality of purchased food products. The manufacturer is convinced that he did everything to provide product that is safe for human health. A characteristic property of these systems is integration of quality management and food safety, and taking over the supervision and control throughout the food chain according to the principle "from farm to fork"(from farm to fork, from stable to table).

Key words: animal traditional products, promotion, quality systems

Introduction

In promotion of animal products should be used trendy expression “authentic food”. As authentic food can be describe e.g. very attractive products, characteristic only for specific country of specific region of country e.g. regional honey etc. In “authentic food” production very important and specific role plays local animal breeds and plants, which guarantee specific properties of final products. Local breeds usually outgoing in production range to new, intensive breed of animals, but products from them have better quality. It is another chance gave this breed of animals for subsistence and development. It is another chance for progress in cattle husbandry (both dairy and beef), poultry, fishery and beekeeping and turn consumers attention on good quality products.

Promotion and marketing are needed for every product, even for the best product on market, where there is fierce competition. About how important is quality of animal products show many quality control systems. Example of this in Poland can be pork quality system PQS, created by Polish Pig Breeders and Producers Association “POLSUS” and “Polish Meat” Association and signed by Minister of Agriculture and Rural Development as national quality control system. PQS is a comprehensive system of production of high quality pork. 12 June 2007 Minister of Agriculture and Rural Development conceded system “Quality Tradition” as national quality control system. This system is used to highlighting food products, especially traditional products of high quality. To this system are included only products of high quality resulted from its traditional character, characterised by specific quality or another properties distinctive them from another products from same category.

Materials and Methods

As traditional composition, traditional production process, traditional character are taken into consideration products with 50 years origin (two generations). As traditional breeds are describe breeds reared before 1956. Traditional product is a product which quality and precious properties results from use of traditional production methods, which are describe as documented methods used at least 25 years. This is product created from traditional raw materials or characterised by traditional composition or production method or processing consistent with traditional method. The product that fulfil the above requirements may be included on the List of Traditional Products, which was established according to section 8 of the Act from 17 December 2004 about the registration and protection of names and designations of agricultural products and foodstuffs and on traditional products *(Journal of Laws of 2005 . No. 10, poz.68)*. In the European Union since 1992, operates a system of protection, identifying and highlighting high quality agricultural products and foodstuffs. These products owe their uniqueness a specific geographical origin and traditional production method. The primary and direct cause of the creation in EU legislation separate regulation regarding these products, was to provide them comprehensive protection that would prohibit the use of false names referring to the traditional method of production or indicating the place where is produced. No less important was the creation of a mechanism that would give rural residents the possibility to increase production and promote their products, thereby contributing to growth and prosperity in the areas where the product is produced. Manufacturers of regional and traditional, except the right to protect the name, they can also place symbols on the packaging showing the uniqueness of the product. Graphic of these symbols is consolidated and bound in all EU countries. Their recognition among European consumers reaches several percent. Another argument, encouraging to create regulations binding in the whole European community devoted to regional and traditional products has been a success, created in the first half of the twentieth century, the French system of protection of geographical names. The number of registered products in the French system, like the value of their sales increased steadily for decades. French manufacturers of products having confirmed a geographical indication could count on the enormous interest consumers who are looking for unique products and were willing to pay for correspondingly higher prices.

Results and Discussion

The European Union using a French solution adopted two regulations concerning geographical indications and designations of origin and regulation

certificates specific character that introduced to the European law the three instruments for the protection and highlight regional and traditional specialties. The nature and procedure for registration of food products because of the origin or the traditional way of processing are regulated by the European Council:

- Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs.
- Council Regulation (EC) No 509/2006 on agricultural products and foodstuffs as guaranteed traditional specialties.

On 5 January 2009 the Act amending previous Act regarding registration and protection of names and designations of agricultural products and foodstuffs and on traditional products and amending certain other acts *(Journal of Laws No. 216 item. 1368, 5 December 2008)*. The Act defines i.a. tasks of Inspection of Quality of Agricultural and Food Products for products with protected designation of origin (PDO), protected geographical indications (PGI) or as traditional specialties guaranteed (TSG), in particular rules for the implementation: check compliance of the production process of products PDO, PGI and GTS specification ; supervision of certification bodies authorised to conduct inspection, issuing and revoking certificates of conformity of production of PDO, PGI and GTS with specification.

Protected Designation of Origin-
if the name of the product, quality, and the whole production technology process is tightly and objectively linked with the origin

The name of an agricultural product or foodstuff may be registered as a "Protected Designation of Origin" (called Protected Designation of Origin - PDO) in the following cases:

• name of the product should use the name of a region, specific place or, in exceptional cases, a country where the product is produced (eg, "Miel de La Alcarria" - Spanish honey from the province Alcarria)

• production of raw materials, processing of raw materials and its preparation for market, or the whole technological process, should take place in the area to which the name refers

• quality of the product or its characteristics should be mainly or exclusively associated with the geographical environment, where the product is produced, ie the product quality should be the result of its origin

• in exceptional cases, as a Protected Designation of Origin may also be registered, some traditional, not geographical names designating an agricultural product or foodstuff (eg, Greek cheeses, "Sfela" or "Kasseri")

For registration of designation of origin can therefore applied manufacturers of products, whose quality is very closely and objectively linked to the origin of the product of the geographical area referred to by name

Protected Geographical Indication -
if the product has unique attributed thanks to its geographic origin and at least one of the stages of the production process takes place in the area referred to by name.

The name of an agricultural product or foodstuff may be registered as a "Protected Geographical Indication" (PGI) in the following cases:

- name of the product should use the name of a region, specific place or, in exceptional cases a country where the product is produced (eg, Jambon d'Ardenne - Ardennes ham)
- the product has a special specific quality, reputation, is acclaimed or have other characteristics attributable to its geographical origin
- product quality may be the result of both, natural and human factors - climate, vegetation, topography, soil, or the uniqueness of local producers skill, methods and traditions of production (the local know-how)
- there is a link between the product and the area from which the product originates; this relationship, however don't need to be as strong as in the case of Protected Designation of Origin - it is required that at least one (not all) of the stages of the entire production process - ie production of raw materials, manufacture the product or preparation for market - took place in an area referred to by name

Traditional Speciality Guaranteed-
if the product has features distinguishing it from other products in the same category and the specific character of the product comes with its traditions.

Agricultural product or foodstuff may be issued "certificate of specific character" (in English Traditional Speciality Guaranteed-TSG) in the following cases:

- The product must have a "specific character" - this means that the product has a feature or set of characteristics that distinguish it from other products similar to it, or belonging to the same category, the specific nature cannot be restricted to qualitative or quantitative composition or method of production laid down in national or community legislation

- The name of the product must be specific in itself (eg Spanish cakes "Panellets") or express the specific character of the agricultural product or foodstuff (eg, Belgian beer "Lambic", which the first phase of fermentation takes place with the participation of Brettanomyces yeast and Bruxellensis Lambicus)
- The product has a traditional character, it means that is produced using traditional raw materials or characterised by a traditional composition or methods of production or processing reflecting a traditional manufacturing method

Only manufacturers who produce the products according to specifications may use the Community symbols. They can be placed both on products and in advertising and promotional materials. Way they are used depends on the manufacturers. They are limited only by the provisions relating to colour of the symbol Products registered or listed by the European Commission in the Register of Protected Designations of Origin and Protected Geographical Indications Registry, or the Traditional Specialities Guaranteed. In accordance with the provisions of the registered names are protected against:

- Currently (as at 20 June 2011) on the List of Traditional Products created by the Ministry of Agriculture and Rural Development we can find 881 product, but as a product with Protected Designation of Origin, Protected Geographical Indication or a Traditional Speciality Guaranteed 28 Polish products are marked

Polish farmers involved in food quality systems can count on financial support, which is particularly important because the market for a high, proven quality in Poland is growing rapidly.

We believed that the traditional Serbian products such as sausages from pigs breed Mangalica, Leskovačka pljeskavica, Šarska pleskavica, Hajdučka pljeskavica, Karadjordjeva snicla (Karađorđeva šnicla), ćevapčići, vešalica, ćulbastija, Leskovačka mućkalica, Vranac, Rakija slivovica aspire to the EU system of protection, identifying and highlighting high quality agricultural and food products.

Conclusion

Promotion of animal products on the market requires a strong and recognizable brand, as often the consumer is not aware of the origin of the product they are buying. A brand is a name, term, symbol, design or combination thereof, designed to identify goods or services to sellers or their groups and their awards from the competition. Mark identifies the vendor or manufacturer. Top brands

represent a guarantee of quality and outside the sphere of material can enter the realm of psychic experience of the consumer.

Conducive to building a strong brand clusters. Clusters are geographic concentrations of interconnected companies, specialized suppliers, service providers, firms in related industries and associated institutions in particular fields, competing with each other but also cooperating.

Promocija proizvoda životinjskog porekla, kao prilika za dalji razvoj stočarstva

W. Migdal, B. Živković, Ł. Migdal

Rezime

Kako bi se osigurala bezbednost mesa i mesnih proizvoda, kao i potreba ponovljivosti kvaliteta proizvoda u celom prehrambenom lancu, sirovine proizvođači su dužni osigurati odgovarajuće uslove za proizvodnju (i ratarskih useva i stočarstva). To obavezuje implementaciju i primenu sistema kao što su: dobra poljoprivredna praksa (GAP), dobra proizvođačka praksa (GMP), dobra higjienska praksa (GHP), a za posredničke firme koje učestvuju u trgovini sirovinama obavezno sprovođenje GHP i GMP sistema, kao i HACCPa *(Hazard Analysis and Critical Control Points)*, QACP *(Quality Assurance Control Points)*, ISO 9001, ISO 22000. Godine 1987. osnovana je ISO9000 serija standarda Međunarodne organizacije za normizaciju za odnose upravljanja kvalitetom i osiguranja kvaliteta. U ovom sistemu kontrola kvaliteta je kontinuirani proces i odvija se na svakom koraku od dizajna do proizvodnje do otpada. Primena sistema upravljanja zahteva rekonstrukciju i širenje protoka informacija između kanala za preradu (objekata za preradu mesa) i dobavljača sirovina (uzgajivači životinja, klanice) u vezi pravila pripreme i implikacija koje se odnose na hranu i lekove. Za ispravno funkcionisanje sistema upravljanja kvalitetom potrebno je osigurati najviši kvalitet u celom lancu hrane, tj. od farme do stola. Analiza opasnosti i kritičnih kontrolnih tačaka (HACCP) je sistem upravljanja kvalitetom, za proces proizvodnje hrane, koji je uveden EU Direktivom br 92/46 i sprovodi se u Poljskoj, uglavnom, na način kako bi proizvođač mogao da postane pouzdan dobavljač hrane na EU tržištu. Uskladjivanje poljskih zakona sa propisima Evropske unije zahteva mnoge aktivnosti, uključujući širenje načela GMP i implementaciju HACCP sistema u prehrambenoj industriji (prema Direktivi EU 93/94/ECC u higijeni hrane). HACCP sistem se smatra najdelotvornijim, najefikasnijim alatom u osiguravanju visokog standarda higijene u proizvodnji i preradi hrane. Generalno, HACCAP sistem se može opisati kao procedura za identifikaciju opasnosti za

zdravlje i rizika od njihovog pojavljivanja u svim fazama proizvodnje hrane i distribucije. To je sistem koji kontroliše i štiti rizike relevantne za potrošače sigurnost i zaštitu zdravlja. HACCP štiti interese potrošača dajući mu sigurnost i visok zdravstveni kvalitet kupljenih prehrambenih proizvoda. Proizvođač je uveren da je učinio sve da proizvede proizvod koji je siguran za ljudsko zdravlje. Osobina tih sistema je integracija upravljanja kvalitetom i sigurnosti hrane, te preuzimanje nadzor i kontrole kroz lanac hrane po principu "od polja do trpeze" (od farme do tanjira, od štale do stola).

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WELFARE PROBLEMS IN DAIRY CALVES

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Abstract: The most significant welfare problems in dairy calves in intensive rearing conditions have been investigated in this paper. These problems mainly result from inadequate intake of colostrum (separation from the dam), inadequate ventilation (resulting in inappropriate airflow, low or high temperatures, high humidity and poor air quality), poor floor conditions (wet floor, without bedding), inadequate monitoring of health, exposure to pathogens causing respiratory and gastrointestinal disorders and occurrence of iron deficiency. In addition, on cattle farms in our country welfare problems in calves originate from continuous restocking (no "all in - all out") and mixing calves from different sources, as well as insufficiently balanced solid food, insufficient access to water and generally poor response of farmer to health problems, especially necessary dietary changes. A special welfare problem is insufficient floor space allowance which results in calf's discomfort.

Key words: calves, welfare, problems

Introduction

The organism of calves in the intensive rearing conditions is influenced by numerous unfavourable factors that may interfere fulfilling their basic needs. These factors may disturb normal breathing, feeding and watering, the development of the digestive tract, rest and sleep, behaviour, social contacts, thermoregulation and self-grooming of the calves. In addition, adverse factors can affect the appearance of fear, pain, injuries and diseases of calves.

Many complex factors contribute to the welfare of calves in commercial dairy farms, including housing and environment, nutrition programs, health, breeders, calves and procedures with dynamic changes in the size of the herd. Many data about these factors were presented in publications by *Broom and Fraser (2007), Philips (2002)*, *Rushen et al. (2008)*, *Stull and Reynolds (2008)*. Provision and

assessment of the welfare of calves in commercial dairy farms is a complex scientific challenge, especially because the terminology and assess factors that contribute to the welfare of calves are not clearly defined *(Veissier, 1996; Vasseur et al., 2009; Vasseur et al., 2009a)*.

The aim of this paper is to assess the welfare indicators of calves on commercial dairy farms in our country and the basis for the consideration of the most common failures in maintaining the welfare of these categories of cattle.

Materials and Methods

The investigations on 18 composite indicators of calves' welfare on 15 commercial dairy farms were conducted. The sizes of the farms were from 50 to 600 cows, with the corresponding number of calves. Each indicator was tested including a detailed assessment of a number of parameters according to the methodology of *Cziszter et al. (2009)* i *EFSA (2006)* and rated using methodology of *Hristov et al. (2010a)*. To asses welfare of calves at observed farms following scale of grades: (5 - excellent, 4 – very good, 3 - good, 2 - sufficient, 1 - insufficient, with resources for improvement, and 0 - insufficient, with no resources for improvement) and rating scale (0-1,99 insufficient, 2,00-2,49 sufficient, 2,5-3,49 good, 3,5 – 4,49 very good and 4,5 – 5,00 excellent) were defined.

Assessment includes a written plan of welfare protection, management, competence of professionals and breeders, veterinary and zootechnical practices, maternal care, behavioural indicators (satisfaction of the behaviour system of calves, the ability to search and social contact), nutrition (colostrum, milk and solid foods), access to water, rearing conditions (space, microclimate and sanitary facilities; ventilation, flooring, light), health (respiratory and gastrointestinal disorders), physiological indicators (clinical values, iron deficiency), continuous use of facilities (without "all in-all out" system), mixing calves from different sources and production indicators of the welfare of calves.

Results and Discussion

The results of assessment of calves' welfare indicators on commercial cattle farms are shown in Table 1.

The research results, presented in Table 1, showed that there are no written plans for protecting the welfare of calves on the three observed farms. On other farms, these plans are present, but on the seven farms they are partial and unsystematic, and the remaining five farms they are mostly relevant.

Table 1. Calves welfare indicators assessment

Parameter	0.00-1.99	2.00-2.49	2.50-3.49	3.5 -4.49	4.5 -5.00
Written plans of welfare protection	3	7	3	2	0
Management	3	7	3	2	0
Competences of breeders	0	1	11	3	0
Competences of professionals	0	1	11	3	0
Veterinary and zootechnical practices	0	1	11	3	0
Maternal care	7	5	2	1	0
Behavioural indicators	3	7	4	1	0
Nutrition	3	5	6	1	0
Access to water	2	2	10	1	0
Rearing conditions	2	2	10	1	0
Ventilation	2	2	10	1	0
Flooring	2	2	10	1	0
Light	2	2	10	1	0
Health	3	6	4	2	0
Physiological indicators	3	6	4	2	0
Continuous use of facilities	2	5	4	2	2
Mixing calves from different sources	1	1	1	10	2
Production indicators	3	6	4	1	1

Rating scale: 0-1.99 insufficient, 2.00-2.49 sufficient, 2.5-3.49 good, 3.5 – 4.49 very good and 4.5 – 5.00 excellent.

When it comes to management on the welfare of calves, it was noted that there is unsystematic and fragmented experience of professionals on the ten farms. The management and governance was not adequate on three farms, and on the other seven farms could be described as satisfying, with numerous small failures. Competences of professionals and breeders (knowledge, skills and attitudes about the welfare of calves) on one farm have been assessed as sufficient, and on the remaining farms as good or very good. The study noted that professionals and breeders in general have a solid competence in terms of rearing conditions (space, microclimate and sanitary facilities, ventilation, flooring, lighting), food and watering, healthcare and the satisfaction of the basic behaviour of calves. Stockmen acquire their competence about welfare mainly on farms, without formal training; professionals learn in the respective institutions of higher education. Veterinary and zootechnical procedures related to the welfare of calves are mostly at the appropriate level on all farms, although certain failures were identified in this segment. Quality of space (floor features), microclimate (ventilation, microclimate factors) and the hygienic conditions of rearing (mechanical cleaning, sanitation) that affects the welfare of calves on farms was assessed as inadequate on two farms, sufficient for two and good on 10 farms. Behavioural indicators (satisfaction of all systems of animal behaviour, search and social contact), indicators of health status of calves (respiratory and gastrointestinal disorders), and food (quantity and

quality of colostrum, milk and solid feed) and productivity (weight) are not at appropriate level on three farms.

On the farms where welfare of calves have been assessed as insufficient, omissions are mainly related to the separation of calves from the mother immediately after birth, insufficient quantity and quality of colostrum, inadequate ventilation (inadequate air flow, low or high temperatures, high humidity and poor air quality), bad floor characteristics (wet floor, floor without bedding), inadequate monitoring of the health of calves by breeders and poor hygienic conditions, leading to exposure of calves to respiratory and gastrointestinal disorders.

In addition, problems related to the welfare of calves originating from the continuous use of stable (non-existence system "all in - all out"), as well as under-balanced roughage to concentrate, lack of access to water and farmers generally untimely response to calf health problems, especially in necessary changes in the diet. A special problem of insufficient welfare of calves is surface of individual boxes that result in discomfort to the calves lying and standing.

An important indicator of welfare is the maternal care of calves *(Broom and Fraser, 2007; Flower and Weary, 2003)*. It is known that the connection between the calf and its mother establishes by licking calves at birth. Licking calves stimulate activities which promote the search for tit *(Fraser and Broom, 2007)*. Colostrum intake during the first six hours provides calves protection against infection and with very important nutrients. Good organization of after birth practice and supply calves with adequate amounts of quality colostrum is very important *(EFSA, 2006; Hepola, 2008; Radostits et al., 2007)*. When feeding calves with milk, it is necessary to strictly take care of hygiene and milk temperature. Also, there is need to have in mind that the stomach of a newborn calf is small, so initially calves should be given small amounts of milk in several portions. In addition, immediately after birth, it is essential to do the best for hygiene of delivery space and equipment that is used to for the colostrum feeding which can significantly reduce the contact of calves with pathogenic organisms *(EFSA, 2006; Hepola, 2003; Radostits et al., 2007)*.

The calves are separated from their mothers at an early stage and usually held individually in a small space in crates with preventing contacts with other animals *(Flower and Weary, 2003)*. In the more spacious crates calves spend more time in self-grooming, while in constrained crates have difficulties in changing positions: lying and standing and limb extension during lying *(Howard, 2003)*. Individual rearing disturbs development of social behaviour of calves. Young calves reared in group interact with each other more frequently than those kept individually *(Hepola, 2003; Hepola, 2008)*. Long periods of social isolation lead to disruption of normal social behaviour in calves. Small crates reduce the expression of other behaviours, such as behaviour in the form of playing *(Jensen et al., 1998)*. Spaces for calves must be so constructed that each calf could lie down and stand up, rest and self-groom without difficulty *(DEFRA, 2003)*.

Whether calves are kept in crates or in groups, nutrition has a major impact on the welfare state. The two most common problems are related to the low iron diet and lack of fibres. Food for calves must contain sufficient quantity of iron to provide the average level of haemoglobin in the blood of at least 4.5 mmol/L, and minimum daily ration of roughage for each calf over two weeks in amounts that are increased from 50 g to 250 g per day for calves aged from eight to 20 weeks *(DEFRA, 2003; Broom and Fraser, 2007; EFSA, 2006)*. All calves older than two weeks must have access to sufficient quantities of fresh water. In the case of high ambient temperature or in the case of the disease, calves must be always provided with fresh drinking water. Equipment for feeding and watering must be designed, constructed, installed and maintained in such a way that contamination of food and water is reduced to a minimum *(DEFRA, 2003)*.

The owner or stuff responsible for animals must controlled calves facilities at least two times a day. For any calf showing signs of disease or injury, responsible person must immediately provide the appropriate care, and if the situation does not improve, have to ask the veterinarian for advice as soon as possible. If it is necessary, sick or injured calves have to be separated into appropriate areas with a dry and comfortable bedding *(DEFRA, 2003; EFSA, 2006)*.

Floors have to be smooth but not slippery, preventing injuries of the calves, maiden and installed in away not to cause injury or suffering of standing or lying calves. Floors must match the size and body mass of calves and make a firm, flat and stable surface. Lying surface must be clean and comfortable, with adequate drainage and shall not negatively affect the calves. All calves under two weeks must be provided with adequate bedding. Calves must not be permanently kept in the dark. In order to meet the needs of calves with respect to behaviour and physiology, it must be ensured adequate natural or artificial lighting *(DEFRA, 2003; EFSA, 2006)*.

In the first few days after the birth, some of the biggest problems concerning the welfare of calves are the digestive and respiratory diseases. Temperature and ventilation are closely related to the level of disease and thereby directly affect the welfare of calves. Provision of adequate food enables calves adaptation to a wide range of temperatures, with an upper critical temperature of 20-25° C and lower critical temperature of about 8° C at the age of one week *(Webster, 1984)*.

Conclusion

According performed investigations of welfare of calves on commercial dairy farms could be concluded:

- on the most farms the separation of calves is carried out immediately after birth, which disrupts the establishment of social bonds between mother and calf, and negatively affect the welfare of calves;

- significant failures in welfare protection plans, governance and management concerning the welfare, quality and quantity of colostrum, ventilation and characteristics of the floor were identified;
- weaknesses in monitoring the health of calves by the breeder, and creating unfavourable hygienic conditions leading to exposure of calves respiratory and gastrointestinal disturbances were identified;
- particular problem of the welfare of calves areas is inadequate size of individual crates, resulting in discomfort to the calves lying and standing.

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Problemi dobrobiti kod teladi

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Rezime

U radu su istraženi najznačajniji problemi dobrobiti kod teladi u intenzivnim uslovima gajenja. Ovi problemi su uglavnom posledica neadekvatnog uzimanja kolostruma (odvajanja od majke), neadekvatne ventilacije (dovodi do neodgovarajućeg protoka vazduha, niske ili visoke temperature, visoke vlažnosti i lošeg kvaliteta vazduha), loših uslova poda (vlažan pod, pod bez prostirke), neadekvatnog praćenja zdravlja teladi od strane odgajivača, izloženosti patogenim mikroorganizmima koji izazivaju respiratorne i gastrointestinalne poremećaje i pojave nedostatka gvožđa. Pored toga, na farmama u našoj zemlji problemi dobrobiti kod teladi potiču od kontinuiranog korišćenja staja (ne "sve unutra- sve napolje"), mešanja teladi iz različitih izvora, kao i od nedovoljno izbalansirane čvrste hrane, nedovoljnog pristupa vodi i generalno lošeg odgovora farmera na zdravstvene probleme teladi, a posebno neophodne promene u ishrani. Poseban problem dobrobiti teladi su nedovoljne površine individualnih boksova koje rezultiraju u neudobnosti teladi.

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THE POSSIBILITY OF DAIRY FARMS ISOLATION ASSESSMENT - BIOSECURITY ASPECT

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Abstract: The effects of general and special biosecurity measures to prevent introduction of infectious agents in a dairy farm or its spreading from farm were analysed in details in this paper. Investigations were conducted on five different dairy farm production with different technology and capacity using a questionnaire method *(Hristov and Stanković, 2009)*, as well as scrutinizing their positions in relation to possible sources bio-risks from the near and far away environment. Test results showed that there are serious shortcomings on all observed farms, regarding the possibility of introduction of infectious agents in the production herd, and its possible spread to the environment. Although all fenced and a position of the most of the farms mostly favourable, there are some serious threats to the herd health and farm production, regarding open space and lack of green belt, the uncontrolled presence of wild birds and rodents in facilities and even in feed storage, as well as contact of the employees with other cows not belonging to the farm.

Key words: biosecurity, isolation, dairy, farm

Introduction

Since the early 1900s, it could be found in veterinary texts that there are a cause and effect relationship between animal contact and disease *(Anderson, 1998)* and that livestock producers recognized animal movements as important routes for the disease dissemination *(Woolhouse and Donaldson, 2001)*. No matter of level of reference (individual, herd or farm level), infectious diseases transmission is related to any form of contact, direct or indirect *(Brennan et al., 2008)*.

There are a number of diseases (bovine tuberculosis, foot and mouth disease – FMD, e.g.) are being spread by movement of animals *(Gibbens et al., 2001; Gilbert et al., 2005; Woolhouse et al., 2005)* which was clearly confirmed during the 2001 FMD outbreak in the UK *(Ortiz-Pelaez et al., 2006)*. There is no doubt that all other kind of contacts may also lead to transmission of infectious agents: equipment sharing, movement of people – contractors or visitors, movement of vehicles, contact over/through fences with neighbouring stock, etc. It has been reported that wildlife and even wind can play a role in transmission between contiguous or nearby production units *(Mikkelsen et al., 2003; Woodroffe et al., 2006)*. Therefore, the effects of general and special biosecurity measures to prevent introduction of infectious agents in a dairy farm or its spreading from five observed farm were analysed in details in this paper.

Materials and Methods

Investigations of the effects of general and special biosecurity measures undertaken by stakeholders in order to prevent introduction of infectious agents in a dairy farm or its spreading from farm were conducted on five different dairy farms with different technology and capacity (farm A: 420 cows in loose system of rearing; farm B: 100 cows in loose system of rearing; farm C: 350 cows in loose system of rearing; farm D: 20 cows in tied system of rearing and farm E: 12 cows in tied system of rearing), as well as scrutinizing their positions in relation to possible sources of bio-risks from the near and far away environment.

Biosecurity indicators related to isolation of the farm (position and isolation level, introduction of newly acquired animals into the herd, traffic control, attitude towards visitors, feeding and watering control, manure management, attitude towards other animals, rodents and birds' control, sanitation) were considered and evaluated.

In order to evaluate relevant biosecurity indicators, grades were defined: 5 - excellent, 4 – very good, 3 - good, 2 - sufficient, 1 - insufficient, there are resources for improvement 0 - insufficient, with no resources for improvement, and rating scale: 0-1,99 insufficient, 2,00-2,49 sufficient, 2,5-3,49 good, 3,5 – 4,49 very good and 4,5 – 5,00 excellent, were defined.

SWOT analysis (**S**trength, **W**eakness, **O**pportunity and **T**reat) was performed afterwards, completing data of possibilities of dairy farms isolation as biosecurity aspect of production.

Results and Discussion

In Table 1 are given results of biosecurity indicators evaluation of five farms in respect of estimated isolation level. At two of five observed farms isolation level

was evaluated as insufficient (farms D and E, 1.74 and 1.26, respectively), at two it was good (farms B and C, 3.27 and 3.39, respectively), and only on one it was very good (farm A, 3.63).

Table 1. Results of evaluation of 5 farms in respect of possibilities of their isolation

INDICATOR	FARM				
	A	B	C	D	E
Size of farm (*number of cows*)	420	100	350	20	12
System of rearing	*loose*	*loose*	*loose*	*loose*	*tied*
1. Position and isolation level	3.80	4.22	3.80	1.20	0.60
2. Introduction of newly acquired animals into the herd	4.29	4.21	5.00	1.29	1.25
3. Traffic control	2.30	1.74	1.20	0.70	0.80
4. Attitude towards visitors	1.67	2.13	2.14	1.43	1.43
5. Feeding and watering control	3.00	4.00	3.00	2.43	2.57
6. Manure management	3.00	4.00	5.00	4.00	1.33
7. Attitude towards other animals, rodents and birds control	2.58	2.43	1.67	1.40	0.89
8. Sanitation	3.86	3.47	2.50	1.50	1.21
Average rate of the farm	3.63	3.27	3.39	1.74	1.26
Total rate of all 5 farms	2.60				

Generally, farms D and E, located inside of human settlements, were rated as insufficient (1.20 and 0.60, respectively), as well as introduction of newly acquired animals into the herd (1.29 and 1.25). These farms are rather small and eventual loses caused by disease would be tolerable.

As the greatest weakness was recognized the traffic control, because it was rated as insufficient at four of five farms (farms B, C, D and E: 1.74, 1.20, 0.70 and 0.80, respectively), attitude towards visitors on three farms (farms A, D and E: 1.67, 1.43 and 1.43, respectively) as well as the attitude towards other animals, rodents and birds control on farms: C, D and E (1.67, 1.40 and 0.89, respectively). Sanitation was rated as very good only at one farm (farm A, 3.86) and good at farms B and C (3.27 and 3.39), but insufficient at farms D and E (1.74 and 1.26).

Although the average grade for all observed farms was good (2.60), general attitude of farms owners towards necessity to isolate their production unit is generally problematic, partly because dairy production is mainly outdoor oriented, so they conclude that contact with other farms, people and other species are inevitable. Studies conducted in The Netherlands, California and New Zealand have identified and quantified these contacts over time, particularly with regard to

the potential spread of FMD *(Brennan et al., 2008)*. The number of contacts varies greatly when considering characteristics such as type of enterprise, size of farm and number of animals on farm *(Bates et al., 2001; Nielen et al., 1996; Sanson et al., 1993)*, illustrating the structural complexity and heterogeneity of the contacts that exist between farms. Cattle, particularly calves, tended to be transported individually. Social visits are responsible for a large number of contacts, and in 25% of these visits the persons had contact with the farm animals, causing the contact to be a high risk for spread of FMD. Cattle farms and mixed pig and cattle farms have more contacts than pig farms, respectively, but the contact pattern would be expected to change drastically after the declaration of an outbreak of foot-and-mouth disease, because of the movement restrictions that would be imposed *(Nielen et al., 1996)*. Measure of importance of these facts was presented in paper by van *Schaik et al. (1998)*. In this paper, Bovine Herpes Virus 1 (BHV1)-positive farms were found to be situated closer to other cattle farms and had more (professional) visitors in the barn that used farm clothing less often and purchased cattle and participated in cattle shows more often, compared with the BHV1-negative farms.

In addition, all ways of transport are expanding in reach, speed of travel and volume of passengers and goods carried, so pathogens and their vectors can now move further, faster and in greater numbers than ever before. Three important consequences of global transport network expansion are infectious disease pandemics, vector invasion events and vector-borne pathogen importation *(Tatem et al., 2006)*. This means that the role of the visitors from other countries as potential pathogen carriers increases and must not be neglected.

Nevertheless, there is permanent problem on dairy or beef cattle farms is misunderstanding or even deliberate neglect of the importance of systematic application of biosecurity measures by employees and/or owners, in respect of isolating and layout of individual buildings, the introduction of newly acquired animals in the herd, footbaths functioning, as well as the technological way of doing repetitive tasks such as feeding or milking, use of medical materials and disposal of carcasses. Procedures for sanitation facilities, resources and animals are often not respected, as well as managing data related to the envisaged and applied biosecurity measures *(Stanković et al., 2010a; Stanković et al., 2010b; Stanković et al., 2011)*.

Obtained data revealed that feeding and watering could be rated as good on all observed farms, but problem of mixed use of equipment and vehicles for both feedstuffs and waste managing and transport might introduce pathogens into herd anytime, especially for younger categories.

Manure management is rather good organized on all farms. According to *Oliver et al. (2005)*, good manure management practices are critical in assuring dairy farm hygiene. Identification of on-farm pathogen reservoirs could aid with

implementation of farm-specific pathogen reduction programs. Manure, lagoon water and bedding constituted areas have to be of major concern on dairy farms.

Conclusion

According to obtained and analysed data regarding possibility of commercial farms isolation as aspect of biosecurity it could be concluded:
- although all fenced and a position of the most of the farms mostly favourable, there are some serious threats to the herd health and farm production, regarding open space and lack of green belt, the uncontrolled presence of visitors, birds and rodents in facilities and feed storage areas;
- general attitude of farms owners towards necessity to isolate their production unit is usually problematic, partly because dairy production is mainly outdoor oriented, so they conclude that contact with other farms, people and other species are inevitable;
- visitors policy and traffic control, as well as sanitation procedures both at the gate and on the farm are the key of good herd health status and successful production.

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Procena mogućnosti izolacije farmi muznih krava - biosigurnosni aspekt

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Rezime

U radu su detaljno analizirani efekti preduzetih opštih i posebnih mera biosigurnosti koji se odnose na sprečavanje unošenja infektivnog materijala u farmu muznih krava ili njegovog širenja sa farme. Ispitivanja su obavljena na 5 farmi muznih krava različite tehnologije proizvodnje i kapaciteta metodom upitnika *(Hristov i Stanković, 2009)*, kao i sagledavanjem položaja farme u odnosu na moguće izvore biorizika u bližoj i daljoj okolini. Rezultati ispitivanja ukazuju da na svim posmatranim farmama postoje ozbiljni nedostaci u pogledu mogućnosti

unošenja infektivnih agenasa u proizvodni zapat, ali i njegovog mogućeg širenja na okolinu.

Iako su sve ograđene, a položaj većine ispitivanih farmi uglavnom povoljan, otvorenost prostora i nedostatak zelenog pojasa, nekontrolisano prisustvo divljih ptica i glodara u objektima za držanje krava pa čak i smeštaj hrane, kao i kontakt zaposlenih sa drugim govedima koja ne pripadaju farmi predstavljaju ozbiljnu pretnju po zdravstveno stanje zapata i proizvodnju farme.

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REGRESSION ANALYSIS OF MILK PRODUCTION TRAITS IN SIMMENTAL COWS

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Abstract: The relationship between milk production traits over whole lactations was evaluated across three generations of Simmental cows, i.e. between daughters, dams and grand dams, by a phenotypic regression analysis with whole lactation traits in the daughter generation being used as the dependent variables (x_1), and those in the dam and grand dam generations being used as the independent variables (x_2 and x_3). The results were obtained from a sample of 1170 daughters and as many dams and grand dams. The significance of the partial regression coefficients b_2 and b_3 was separately evaluated by a t-test. An analysis of variance was used to estimate the significance of the simultaneous effect of the production traits of dams and grand dams on the milk production achieved in the daughter generation. The calculated value of the partial regression coefficients for the whole lactation production traits across three generations (grand dams, dams and daughters) and their statistical significances determined by the t and F tests, as well as the regression equations used, suggested that the effect of the grand dam generation on the milk production traits in granddaughters was substantially lower than the effect of dams. The calculated partial regression coefficients (b_2 and b_3) were positive and statistically very significant ($P<0.01$), excepting the regression coefficients b_3 for lactation length and b_2 for milk fat content that were not statistically significant ($P>0.05$). A very significant change ($P<0.01$) was observed in all production traits in the daughter generation as simultaneously affected by the traits in the dam and grand dam generations

Key words: Simmental breed, production traits, generation, regression.

Introduction

The heritability of milk production traits of 25%, continuous breeding for their improvement and the enhancement of raising conditions lead to both yield increases and the relationship between the traits across generations. However, the

relevant literature on the relationship between production traits across generations is rather scarce.

Yield increases across generations as being facilitated by continuous breeding work and improving raising conditions, primarily nutrition and care, have been reported by international authors *(Rycken, 1996, 1997, 1998)*, whereas Serbian authors observe declining trends in milk and milk fat yields across generations in imported breeding heifers as being primarily induced by poorer raising conditions and earlier exposure to breeding as compared to the situation in Western European developed countries (Germany, Switzerland, Austria) from which they were imported *(Bogdanovic et al., 2008; Lazarevic et al., 1984; Nenadovic et al., 1986; Petrovic M.M.,1997, 2008; Petrovic D.M., 2008; Petrovic D.M. et al., 2009; Pantelic et al., 2011)*.

The above suggests that more substantial increases in milk performance in Serbian spotted Simmental dairy cattle can be achieved through breeding work, by using superior sire bulls and via timely exposure to breeding along with continuous improvements in raising conditions as compared to the imported Simmental breeding heifers.

In their study on the milk production potential and performance across generations of Simmental cows in Poland, Tarkovski and *Trautman (1997)* attributed the increase in milk production across generations to the use of Simmental sires imported from Germany, Austria and Switzerland.

Analysing the milk performance of Simmental cows in Switzerland during 1995-1996, 1996-1997 and 1997-1998, *Rycken (1996, 1997, 1998)* indicated that milk performance improvement across generations was a result of improvements in both the genetic potential of the bull sires and the raising conditions used.

Materials and Methods

The relationship between milk production traits over whole lactations was evaluated across three generations of Simmental cows, i.e. between daughters, dams and grand dams, by a phenotypic regression analysis with whole lactation traits in the daughter generation being used as the dependent variables (x_1), and those in the dam and grand dam generations being used as the independent variables (x_2 and x_3). The results were obtained from a sample of 1170 daughters and as many dams and grand dams.

Milk production traits of whole lactations, as assessed by regression analysis, included the following:

- lactation length (WLL), (days),
- milk yield over whole lactations (MYWL), (kg),
- milk fat content over whole lactations (MFCWL), (%),
- milk fat yield over whole lactations (MFYWL), (kg) and

- 4% FCM yield over whole lactations (4%FCMWL), (kg).

The regression analysis employed involved the calculation of the $a_{1.23}$ parameters (a) and the partial regression coefficients $b_{12.3}$ (b_2) and $b_{13.2}$ (b_3) using the following general linear multiple regression model involving three variables:

$$\hat{X}_1 = a_{1.23} + b_{12.3}X_2 + b_{13.2}X_3 \quad \text{or} \quad \hat{X}_1 = a + b_2X_2 + b_3X_3$$

The partial regression coefficients $b_{12.3}$ (b_2) and $b_{13.2}$ (b_3) were calculated using the following system of equations:

$$\sum_{i=1}^{n} x_{1i}x_{2i} = b_2\sum_{i=1}^{n} x_{2i}^2 + b_3\sum_{i=1}^{n} x_{2i}x_{3i}$$

$$\sum_{i=1}^{n} x_{1i}x_{3i} = b_2\sum_{i=1}^{n} x_{2i}x_{3i} + b_3\sum_{i=1}^{n} x_{3i}^2$$

Unknown partial regression coefficients (b_2 and b_3) were computed using determinants:

$$b_2 = \frac{D_{b_2}}{D} \quad \text{and} \quad b_3 = \frac{D_{b_3}}{D}$$

The third constant of linear multiple regression a, as the average initial level, was calculated using the formula:

$$a = \overline{X}_1 - b_2\overline{X}_2 - b_3\overline{X}_3$$

The significance of the partial regression coefficients b_2 and b_3 was tested using the following t-test:

Ho: $b_2 = 0$ Ho: $b_3 = 0$

$$t = \frac{|b_2|}{S_{b_2}} \qquad t = \frac{|b_3|}{S_{b_3}}$$

Where:

X_1, X_2, X_3 – value of whole lactation variable traits of daughters, dams and grand dams,

$a_{1.23}$ or a – constant value, representing the average initial level of whole lactation traits,

$b_{12.3}$ or b_2 and $b_{13.2}$ or b_3 – partial regression coefficients,

$\sum_{i=1}^{n} x_{1i}x_{2i}, \sum_{i=1}^{n} x_{2i}^2, \sum_{i=1}^{n} x_{2i}x_{3i}, \sum_{i=1}^{n} x_{1i}x_{3i}, \sum_{i=1}^{n} x_{2i}x_{3i}, \sum_{i=1}^{n} x_{3i}^2$ - corrected sums of squares and intermediates in three generations of cows,

D, D_{b_2}, D_{b_3} - determinants,

S_{b_2}, S_{b_3} - standard errors of the regression coefficients.

The hypothesis that the regression coefficients b_2 and b_3 were equal to unity (Ho: $b_2 = b_3 = 0$) was checked by the analysis of variance method using the Statistica statistical software for Windows Release 5.0. This analysis was used to determine F_{exp} values i.e. the significance of the simultaneous effect of production traits of dams and grand dams on the level of production achieved in daughters.

Results and Discussion

Regression analysis was employed to calculate the parameters $a_{1.23}$ (a) and partial regression coefficients $b_{12.3}$ (b_2) and $b_{13.2}$ (b_3). The significance of the coefficients was evaluated by a t-test (Table 1). An analysis of variance (F test) was used to estimate the significance of the simultaneous effect of the production traits of dams and grand dams on the traits of daughters. The results obtained are given in Tables 2 through 5.

Table 1. Regression analysis of whole lactation traits between three generation of cows (daugher, dam and grand dam)

Traits	Parameter $a_{1.23}$	Partial regression coefficients		Multiple regression equation
		$b_{12.3}(b_2)$	$b_{13.2}(b_3)$	
WLL, days	244.156^{**}	0.135^{**}	0.056^{ns}	$\hat{X}_1 = 244.156 + 0.135X_2 + 0.056X_3$
MYWL, kg	2856.296^{**}	0.193^{**}	0.073^{**}	$\hat{X}_1 = 2856.296 + 0.193X_2 + 0.073X_3$
MFCWL, kg	121.050^{**}	0.146^{**}	0.044^{*}	$\hat{X}_1 = 121.050 + 0.146X_2 + 0.044X_3$
MFYWL, %	2.973^{**}	0.0003^{ns}	0.222^{**}	$\hat{X}_1 = 2.973 + 0.0003X_2 + 0.222X_3$
4%FCMWL, kg	2955.519^{**}	0.168^{**}	0.054^{**}	$\hat{X}_1 = 2955.519 + 0.168X_2 + 0.054X_3$

N.S. - $P > 0.05$; * - $P < 0.05$; ** - $P < 0.01$;

lactation length (WLL), milk yield over whole lactations (MYWL), milk fat content over whole lactations (MFCWL), milk fat yield over whole lactations (MFYWL) and 4% FCM yield over whole lactations (4%FCMWL).

The calculated coefficient b_2, as shown in Table 1, indicates that a one-day increase in whole lactation length in dams resulted in a 0.135-day increase in lactation length in daughters with the lactation length in grand dams remaining unchanged. The partial regression coefficient b_3 shows that the whole lactation length in daughters increased by 0.0056 days with a one-day increase in lactation length in grand dams, with the lactation length in dams remaining unchanged.

The analysis of the significance of the partial regression coefficients, as determined by the t-test, reveals that the lactation length in daughters was statistically very significantly ($P<0.01$) affected by whole lactation length in dams and non-significantly ($P>0.05$) by whole lactation length in grand dams.

The analysis of variance (Table 2) suggests very significant changes ($P<0.01$) in whole lactation length in daughters as affected simultaneously by lactation length in dams and grand dams ($F_{exp}=14.77797^{**}$).

Table 2. Analysis of variance on the simultaneous effect of whole lactation length in dams and grand dams on the expression of the trait in daughters

Sources of variation	Sums of squares (SS)	Degrees of freedom (df)	Mean squares (MS(SS/df))	F_{exp}
Regression	104012	2	52006.02	14.77797^{**}
Error	12352250	3510	3519.16	
Total	12456262			

N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01; *** - P < 0.001;

The increase of 0.193 and 0.168 kg in milk yield and 4% FCM yield, respectively, in daughters was associated with each kilogram of the yield increase in dams, with the yield in grand dams remaining unchanged (Table 1). The milk yield in daughters was substantially less affected by the milk performance of grand dams. The one-kilogram increase in milk and 4% FCM yields in grand dams induced an increase of 0.073 and 0.054 kg in the respective yields in daughters, with the milk performance of dams remaining unchanged.

The significance of the partial regression coefficients in milk yield and 4% FCM yield, as determined by the t-test, was very high ($P<0.01$).

Very significant changes ($P<0.01$) in both milk yield and 4%FCM yield in daughters were also induced by the simultaneous effect of milk and 4% FCM yields in dams and grand dams ($F_{exp}=58.81113^{**}$ and $F_{exp}=41.96612^{**}$), as suggested by the analysis of variance presented in tables 3 and 4.

Table 3. Analysis of variance on the simultaneous effect of whole lactation milk production traits in dams and grand dams on the milk performance of daughters

Sources of variation	Sums of squares (SS)	Degrees of freedom (df)	Mean squares (MS(SS/df))	F_{exp}
Regression	1.409118E+08	2	70455892	58.81113**
Error	4.204989E+09	3510	1198003	
Total	4.345901E+09			

N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01; *** - P < 0.001;

Table 4. Analysis of variance on the simultaneous effect of the 4% FCM yield over whole lactations in dams and grand dams on the corresponding yield in daughters

Sources of variation	Sums of squares (SS)	Degrees of freedom (df)	Mean squares (MS(SS/df))	F_{exp}
Regression	9.273446E+07	2	46367231	41.96612**
Error	3.878104E+09	3510	1104873	
Total	3.970839E+09			

N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01; *** - P < 0.001;

The milk fat yield in daughters was very significantly (P<0.01) and significantly (P<0.05) affected by the yield in dams and grand dams. Namely, the milk fat yield in daughters showed a 0.146 kg increase as a result of a 1 kg increase in dams, with the yield of grand dams remaining unchanged (b_2=0.146). The 1 kg increase in milk fat yield in grand dams induced a 0.044 kg increase in daughters with the yield in dams remaining unchanged (b_3=0.044).

The analysis of variance given in Table 5 suggests very significant changes (P<0.01) in the milk fat yield of daughters as simultaneously affected by the milk fat yield of both dams and grand dams (F_{exp}=31,50756**).

Table 5. Analysis of variance on the simultaneous effect of milk fat yield over whole lactations in dams and grand dams on the milk fat yield in daughters

Sources of variation	Sums of squares (SS)	Degrees of freedom (df)	Mean squares (MS(SS/df))	F_{exp}
Regression	106358	2	53178.91	31.50756**
Error	5924228	3510	1687.81	
Total	6030586			

N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01; *** - P < 0.001;

The milk fat content in daughters increased 0.0003% with each percent of increase in dams, with the content in grand dams remaining unchanged. The effect was not statistically significant (P>0.05). Conversely, the effect of the milk fat content in grand dams on that in daughters, with the content in dams remaining unchanged, was

highly significant (P<0.01). Moreover, the 1% increase in the milk fat content in grand dams resulted in a 0.222% increase in daughters.

Table 6. Analysis of variance on the simultaneous effect of milk fact content over whole lactations in dams and grand dams on the content in daughters

Sources of variation	Sums of squares (SS)	Degrees of freedom (df)	Mean squares (MS(SS/df))	F_{exp}
Regression	5.41486	2	2.707431	117.7837^{**}
Error	80.68248	3510	0.022986	
Total	86.09735			

N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01; *** - P < 0.001;

A statistically very significant (P<0.01) change ($F_{exp}=117.7837^{**}$) was observed in the milk fat content in daughters as induced by the simultaneous effect of the milk fat content in dams and grand dams, as suggested by the analysis of variance results given in Table 6.

Conclusion

The calculated value of the partial regression coefficients for the whole lactation production traits across three generations (grand dams, dams and daughters) and their statistical significances determined by the t and F tests, as well as the regression equations used, suggest the following:

- the effect of the grand dam generation on the milk production traits in granddaughters was substantially lower than the effect of dams;
- the calculated partial regression coefficients (b_2 and b_3) were positive and statistically very significant (P<0.01), excepting the regression coefficients b_3 for lactation length and b_2 for milk fat content that were not statistically significant (P>0.05).
- a very significant change (P<0.01) was observed in all production traits in the daughter generation as simultaneously affected by the traits in the dam and grand dam generations

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Korelaciona analiza povezanosti proizvodnih osobina kroz tri generacije krava simentalske rase

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Rezime

Povezanost osobina mlečnosti u punim laktacijama analizirana je između tri generacije krava simentalske rase, odnosno između kćerki, majki i baba, fenotipskom regresionom analizom u uslovima kada su osobine punih laktacija u generaciji kćerki posmatrane kao zavisno (x_1), a osobine punih laktacija u generaciji majki i baba kao nezavisno promenljive (x_2 *i* x_3). Rezultati su dobijeni na uzorku od po 1170 kćerki, majki i baba.

Pojedinačno ispitivanje značajnosti delimičnih regresionih koeficijenata b_2 i b_3 izvršeno je t-testom, a analizom značajnosti istovremenog uticaja proizvodnih osobina majki i baba na ostvarenu proizvodnju kod kćerki izvršena je metodom analize varijanse.

Na osnovu izračunate vrednosti delimičnih regresionih koeficijenata za proizvodne osobine celih laktacija kroz tri generacije (babe, majke i kćerke) i njihove statističke značajnosti određene t i F testom, kao i jednačina regresije konstatovano je da je uticaj generacije baba na proizvodnju unuka znatno manji u odnosu na uticaj kojeg imaju majke. Svi izračunati delimični koeficijenti regresije (b_2 *i* b_3) bili su pozitivni i statistički vrlo značajni ($P<0.01$), izuzimajući koeficijent regresije b_3 kod trajanja laktacije i b_2 kod sadržaja mlečne masti koji nisu bili statistički značajni ($P>0.05$). Značajnost promena svih posmatranih proizvodnih osobina u generaciji kćerki pri istovremenom uticaju tih osobina iz generacije majki i baba bila je vrlo visoka ($P<0.01$).

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PROPERTIES AND COMPOSITION OF CARCASS OF DOMESTIC SPOTTED YOUNG CATTLE OF TWO PRE-SLAUGHTER WEIGHTS

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Abstract: Objective of this research was to determine the slaughter properties and carcass composition of carcass sides derived from young cattle of Domestic Spotted breed, of pre-slaughter weights of 500 (group A) and 600 kg (group B). Heavier cattle had higher share of kidney fat in carcass, whereas the share of offals showed no significant differences. Statistically significant difference was registered only in share of toungue ($P<0.05$), that was higher in cattle of group A. Share of extra/premium (tenderloin), I category (round) and II category (loin, back, shoulder) showed no significant differences between groups of cattle. Statistically significant difference ($P<0.05$) was established in the share of III category carcass parts. Share of forearm and chest was significantly higher in lighter cattle (3.55% and 7.95%) compared to heavier animals (2.89% and 6.33%), whereas the share of subshoulder was considerably lower in group A (6.89%) compared to cattle of group B (9.73%). Data obtained by dissection of main carcass side parts differed significantly in regard to the share of muscle tissue in round and belly, that was significantly higher in cattle of group A ($P<0.05$). Share of fat tissue (subcutaneous and intermuscular) was higher in cattle of group B in almost all carcass side parts, however, statistically significant difference between groups was determined only in round, back, neck and subshoulder. Also, share of bones in chest differed statistically significantly ($P<0.05$) and it was higher in group A (20.09%) compared to cattle of group B (15.52%).

Key words: Domestic Spotted breed, slaughter live weight, carcass, share of tissue

Introduction

In Serbia, production of beef is based on Domestic spotted breed, which is breed of combined production traits. Production of beef since 2001 has been in

slight increase, but it is still unsatisfactory in regard to number of heads, as well as productivity *(Aleksić et al., 2007)*. Beef is on the third place in regard to consumer preference and consumption (21%), after pork (40%) and poultry (31%), which is adequate to structure of consumption in countries with low total meat consumption (below 50 kg per capita) in relation to countries with meat consumption of over 100 kg (SAD, Australia, Canada etc.) where beef has the highest share in the structure of consumption *(Ostojić et al., 2005)*.

Share of tissues, primarily meat, in retail cuts defines thier quality, value and demand on the market. Beef producers are faced with the task of producing economically the kind of cattle that yield a high percent of high-priced, tender, flavourful, juicy retail cuts, but that have only minimum amounts of waste fat trim *(Guenther et al., 1965)*.

Data on content of tissues in main carcass parts that are available in the literature mainly relate to composition of individual anatomical regions/parts of carcass, with high variations depending on the method of cutting of carcass sides, heterogeneity of the material used in the study (bree, sex, age, fattening level, etc.). In that regard, objective of this research was to determine slaughter properties and composition of carcass sides of Domestic Spotted cattle, of average pre-slaughter weights of 500 and 600 kg.

Materials and Methods

Trial was carried out on experimental farm, slaughterhouse and laboratory of the Institute for Animal Husbandry, Belgrade-Zemun, Serbia, on 18 young cattle of Domestic Spotted breed. First group of 9 cattle (A) was slaughtered after reacing an average weight of 500 kg, and the second group (B) wight of 600 kg. During the fattening period the rearing system was free, and food consisted of concentrated feeds, hay and corn grain silage. One day prior to slaughtering animals were deprived food, but had free access to water. Prior to slaughtering, which was carried out according to standard commercial procedures, animals were weighed. After removing the skin and head, front and rear rounds and eviseration, carcasses were placed in cold storage at temperature of 4^0C for next 24 hours. One hour after slaughter and treatment, mass of warm carcass, mass of offals (heart, lungs, liver, kidenys, spleen and tongue) were taken, also of head, tail and kidney fat.

After chilling, the carcasses were measured and split along the vertebral column in two halves, and the left side was used for all measurements. The left side of each carcass was devided into twelve anatomical regions: round, tenderloin, loin part, shoulder, neck, back, chest, subshoulder, ribs, belly, forearm, and foreround, using a standard technique. All carcass parts were separated into dissectible fat (subcutaneous and intermuscular), muscle and bone tissue.

In order to determine the effect of slaughter weight on carcass characteristics, a single-factor analysis of variance was performed using Statistica 7 software (StatSoft, USA). If the effect of slaughter weight was found significant, Tukey's test was used to evaluate the significance of difference.

Results and Discussion

In Table 1, slaughter parameters and shares of slaughtering accompanying products and offals are presented. Dressing percentages of warm and cold carcasses were slightly higher in cattle of group B, but without statistical significance. Stated results for dressing percentage of warm carcass are in accordance with data stated by *Waritthitham et al. (2010)* and *Sanudo et al. (2004)*. Chilling loss of mass was greater in young cattle of group A (2.52%) compared tog roup B (1.72%), however, there were no statistically significant differences in this parameter, probably because high variations in the value of chilling loss within groups and high standard deviations.

Aleksić et al. (2009) stated that share of mass of kidney fat in body mass prior to slaughtering was 0.82% in young cattle with average mass prior to slaughtering of 591.13 kg and 0.79% in cattle with average mass prior to slaughtering of 509.00 kg, which is in accordance with data obtained in this trial.

Table 1. Slaughter properties and share of kindey fat, head, tail and offals of young cattle

Indicator	A	B	t-test
Mass of warm carcass (kg)	277.00 ± 11.01	334.58 ± 15.41	*
Mass of cold carcass (kg)	269.93 ± 15.23	328.77 ± 14.82	*
Dressing percentage of warm carcass (%)	55.31 ± 2.10	56.33 ± 2.39	ns
Dressing percentage of cold carcass (%)	55.49 ± 1.44	57.09 ± 2.75	ns
Chilling loss (%)	2.52 ± 0.28	1.72 ± 0.20	ns
Kideny fat (%)	0.73 ± 0.03	0.81 ± 0.04	*
Head (%)	2.70 ± 0.22	2.63 ± 0.19	ns
Tail (%)	0.22 ± 0.04	0.21 ± 0.05	ns
Offals (%)			
Kidneys	0.18 ± 0.03	0.17 ± 0.03	ns
Liver	1.12 ± 0.11	1.09 ± 0.10	ns
Lungs	0.76 ± 0.08	0.77 ± 0.09	ns
Heart	0.37 ± 0.02	0.32 ± 0.04	ns
Spleen	0.19 ± 0.01	0.19 ± 0.03	ns
Tongue	0.35 ± 0.02	0.28 ± 0.01	*

ns – not significant

* significant at the level of ($P<0.05$)

Share of offals showed no significant differences between groups of cattle (Table 1). Statistically significant difference was established only in share of tongue ($P<0.05$), that wa shigher in young cattle of group A. Data are in accordance with results obtained by *Aleksić et al. (2009)* for young cattle of Domestic Simmental breed.

Increasing slaughter weight from 500 up to 600 kg generally increased body size and weight, carcass size and weight, loin eye area and percentage of commercial prime cuts *(Waritthitham et al., 2010)*. Share of premium cuts (tender loin), I category (round) and II category (loin, shoulder, back) showed no significant differences between groups of cattle (Table 2).

As presented in Table 2, statistically significant difference ($P<0.05$) was established in share of forearm, chest and subshoulder. Share of forearm and chest was significantly higher in lighter (3.55% and 7.95%) cattle compared to heavier (2.89% and 6.33%). Share of subshoulder was considerably lower in cattle of group A (6.89%) compared to group B (9.73%).

According to results obtained by *Aleksić et al. (2009)*, increasing of body mass prior to slaughter of average 509 to 590 kg, statistically significantly ($P<0.05$) increases the share of round/round from 28,39% to 29,86%. Share of round, in this trial, was slightly higher in heavier cattle, however, difference was not statistically significant (Table 2).

Table 2. Shares of main carcass side parts

Carcass side parts (%)	A	B	t-test
Tender loin	1.77 ± 0.11	1.85 ± 0.12	ns
Round	28.07 ± 1.46	29.68 ± 1.37	ns
Parts of II category			
Loin	4.73 ± 0.32	4.72 ± 0.27	ns
Back	5.37 ± 0.41	5.09 ± 0.56	ns
Shoulder	13.25 ± 0.70	13.04 ± 0.52	ns
Total	23.63 ± 0.98	23.21 ± 1.17	ns
Parts of III category			
Round	4.17 ± 0.38	4.03 ± 0.32	ns
Foreshank	3.55 ± 0.11	2.89 ± 0.10	*
Neck	9.87 ± 0.75	9.74 ± 0.63	ns
Breast	7.95 ± 0.38	6.33 ± 0.24	*
Subshoulder	6.89 ± 0.27	9.73 ± 0.49	*
Ribs	7.29 ± 0.59	7.11 ± 1.06	ns
Belly	5.53 ± 1.01	5.79 ± 0.75	ns
Total	46.20 ± 1.22	45.57 ± 2.13	ns

ns – not significant

* significant at the level of ($P<0.05$)

Weight and size of a carcass has a major influence not only on the quantity of the various tissues but also on the size of muscle exposed to cutting and the individual joints prepared from it *(Kampster, 1992)*.

Pdata obtained by dissection of main carcass parts are presented in Table 3. Shares of muscle tissue in round and belly were considerably higher in cattle of group A ($P<0.05$). Share of chest muscle tissue was also higher in lighter animals (66.26%) compared to heavier cattle (62.27%), however, this difference was not statistically significant. *Waritthitham et al. (2010)* state that share of muscle tissue decreasesm and share of fat tissue increases in young cattle of pre-slaughter weight of 600 kg compared to those slaughtered at weight of 500kg. Similar results were obtained by *Sanudo et al. (2004)*.

In the study of the composition of beef carcasses obtained from cattle of average pre-slaughter weight of 597 kg, *Aleksić et al. (2007)* state the share of muscle tissue in round of approx. 86%, in II category cracass parts (loin, back, shoulder) approx. 78%, and in III category parts (neck, subshoulder, forearm, chest, ribs, foreshank) approx. 73%.

Share of fat tissue (subcutaneous and intermuscular) was higher in cattle of group B in almost all carcass side parts, however, statistically significant difference between groups was recorded only for round, back, neck and subshoulder (Table 3). In case of loin, shoulder, chest, ribs and belly, although higher value for the share of fat tissue in cattle of group B was determined, due to great variations of this parameter within group, no statistically signiicant difference was established. Obtained data are not in accordance with conclusion stated by *Keane and Allen (2002)* that extending the feeding time to raise crossbred cattle up to 600 kg live weight does not increase the fat tissue proportion.

Share of chest bones showed statistically significant difference ($P<0.05$) and it was higher in cattle of group A (20.09%) compared to cattle of group B (15.52%). *Sanudo et al. (2004)* in their study show share of bones lower than in cattle of pre-slaughter weight of 550 kg, compared to cattle of pre-slaughter weight of 300 kg, which is in accordance with results presented in this study.

Table 3. Shares of muscle tissue, fat tissue and bones in main carcass side parts

Carcass part/tissue (%)	A	B	t-test
Round			
Meat	85.37±3.32	81.52±2.95	*
Fat	2.91±0.21	5.79±0.33	*
Bone	12.82±0.89	12.35±1.12	ns
Loin			
Meat	71.99±3.14	69.28±5.27	ns
Fat	3.93± 1.26	5.06±2.01	ns
Bone	23.70±2.34	23.22±3.15	ns
Back			
Meat	69.30±2.89	71.71±5.62	ns
Fat	5.45±0.30	7.63±0.54	*
Bone	21.98±2.41	19.28±3.36	ns
Shoulder			
Meat	81.88±2.04	80.58±4.96	ns
Fat	2.11±1.17	4.02±1.28	ns
Bone	16.0±1.04	15.52±1.85	ns
Chest			
Meat	66.26±5.98	62.27±6.82	ns
Fat	13.49±3.28	19.58±4.79	ns
Bone	20.09±1.53	15.52±1.03	*
Ribs			
Meat	78.57±4.83	77.41±7.86	ns
Fat	4.67±1.34	9.02±3.16	ns
Bone	17.55±3.55	12.58±2.35	ns
Neck			
Meat	84.21±4.50	81.23±6.10	ns
Fat	1.22±0.13	7.74±0.71	*
Bone	13.14±2.70	11.30±1.90	ns
Subshoulder			
Meat	75.01±4.20	71.05±5.85	ns
Fat	2.80±0.23	7.22±0.68	*
Bone	20.01±2.36	19.59±4.86	ns
Belly			
Meat	80.33±5.78	74.26±4.54	*
Fat	18.42±4.12	23.01±3.66	ns
Bone	1.19±0.26	1.04±0.22	ns
Round			
Meat	45.34±4.15	43.90±2.17	ns
Fat	1.72±0.77	3.14±1.19	ns
Bone	51.36±4.28	51.08±2.98	ns
Foreshank			
Meat	57.23±4.28	55.26±3.98	ns
Fat	1.36±0.38	3.05±0.59	ns
Bone	40.92±4.66	40.22±2.18	ns

ns – not significant
* significant at the level of ($P<0.05$)

Conclusion

Based on results presented in this paper it can be concluded that Domestic spotted cattle of pre-slaughter weight of 500 and 600 kg, show no significant differences in slaughter properties and shares of accompanying products of the carcass. Statistically significant difference was established in higher share of kidney fat and fat tissue in main carcass side parts in heavier cattle, whereas the lighter animals had considerably higher share of muscle tissue in round and belly.

Acknowledgment

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Osobine i sastav trupa junadi domaće šarene rase različitih težina pred klanje

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Rezime

Cilj ovog istraživanja bio je da se utvrde klanične osobine i sastav polutki junadi domaće šarene rase, zaklanih pri dostizanju prosečne težine od 500 (grupa A) i 600 kg (grupa B). Teža junad su imala veći udeo bubrežnog loja u trupu, dok se udeo iznutrica nije značajno razlikovao. Statistički značajna razlika je pronađena samo u udelu jezika ($P<0.05$), koji je bio veći kod junadi grupe A. Udeo delova ekstra (biftek), I kategorije (but) i II kategorije (slabine, leđa, plećka) nisu se značajno razlikovali između grupa junadi. Statistički značajna razlika ($P<0.05$) je pronađena u udelu delova III kategorije. Udeo podlaktice i grudi bio je značajno veći kod lakših (3.55% i 7.95%) u odnosu na težu junad (2.89% i 6.33%), dok je udeo potplećke bio znatno niži kod junadi grupe A (6.89%) u odnosu na junad grupe B (9.73%). Podaci dobijeni disekcijom osnovnih delova polutke su se statistički značajno razlikovali u udelu mišićnog tkiva u butu i potrbušini, koji je bio značajno veći kod junadi grupe A ($P<0.05$). Udeo masnog tkiva (potkožnog i intermuskularnog) je bio veći kod junadi grupe B kod gotovo svih delova polutke, međutim, statistički značajna razlika između grupa je pronađena samo kod buta, leđa, vrata i potplećke. Takođe, udeo kostiju grudi se statistički značajno razlikovao ($P<0.05$) i bio je veći kod junadi grupe A (20.09%) u poređenju sa junadima grupe B (15.52%).

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ASSOCIATION OF PHENOTYPIC COMBINATIONS Hb/K WITH QUALITATIVE FEATURES OF LAMB PELTS IN THE BOTOSANI KARAKUL SHEEP

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Abstract: The specific production of the sheep belonging to the Botosani Karakul breed is the one of lamb pelts, characterized by various qualitative features (shape and size of hair curls, quality, lustre and colour of hair fibres), conferring nobility to this breed and which distinguish it from other sheep breeds. The present study tries an associative analysis of these traits of lamb pelts with different combinations between haemoglobin phenotypes and potassium phenotypes. The most valuable features, concerning shape and size of hair curls, as well as the quality and lustre of hair fibres are associated with combination of phenotypes HbAB and HK, and the weakest association of these features occur with the phenotypic combination HbBB/LK. The greyish, brown, grey, pink and white colours of the hair fibres are more associated with phenotypic combination HBB/LK, and the lowest frequencies of these colours are found in phenotype HbAB associated with phenotype HK, but this last phenotypic combination is associated to the highest degree with the black colour. The differences among the empirical distributions of qualitative features of the lamb pelts in relation to all phenotypic combinations of the haemoglobin and potassium systems are very significant, fact that recommends the use of these two biochemical genetic systems for the improvement of the Botosani Karakul breed for the lamb pelt production.

Key words: haemoglobin phenotype, potassium phenotype, lamb pelt

Introduction

The great beauty, suppleness and nobility of the lamb pelts of Karakul type, conferred by the colour, elasticity and silky aspect of their hairy cover or by the remarkable design resulted from the curling degree of hair fibres, their arrangement in curls and the curl uniformity, attracted and fascinated man since ancient times. Thus, as an article of fashion, clothing made of Karakul lamb pelts currently satisfies the most

refined clothing preferences of the worldwide, especially those from countries situated in temperate and cold climate zones. The diversity of colour shades, of curl shapes and of their modelling makes from Karakul lamb pelts a natural raw material of animal origin with outstanding physical and biological features which can not be equalled or substituted by the most sophisticated modern synthesis technologies *(Taftă et al, 1997)*.

Besides the determinism of other factors (nutritional, technological, climatic), the lamb pelts of Karakul type have mainly a genetic determinism, for which a large number of protein systems with structural, physiological, enzymatic role or with a cellular and extracellular vehicle function are involved.

In the mentioned context, the approach of association of the different combinations of haemoglobin and potassium types with the qualitative features of the Botosani Karakul lamb pelts did not seem without interest, with a view to use these associations in the selection and improvement processes of this sheep breed for the lamb pelt production.

Materials and Methods

The experimental works were carried out on a randomized population of 381 lambs belonging to the Botosani Karakul breed from the *Research and Production Station for Sheep Breeding, Popauti-Botosani*. The estimation of the main qualitative features of lamb pelt was performed at 24-48 hours after their birth. These features are *(Taftă et al, 1997)* (Figures 1, 2, 3, 4, 5, 6):

- the curl shape, with the characteristics: cylindrical tube, tube+grain, grain, flat tube, varia (heterogeneous and miscellaneous shapes);
- the curl size, with the characteristics: middle, middle-small, small, big;
- the quality of the hair fibre, with the characteristics: silky, normal, rough, soft;
- the lustre of the hair fibre, with the characteristics: intense, good, satisfactory, mat;
- the colour of the hair fibre, with the characteristics: black, greyish, brown, grey, pink, white.

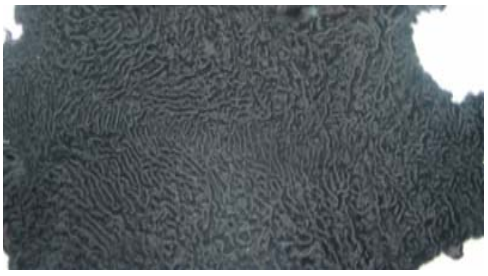

Figure 1. Black lamb pelt

Figure 2. Greyish lamb pelt

Figure 3. Brown lamb pelt

Figure 4. Grey lamb pelt

Figure 5. Pink lamb pelt

Figure 6. White lamb pelt

The identification of haemoglobin phenotypes was made by horizontal electrophoresis method having the starch gel (12.5%), as substrate, and using a solution of Tris(hidroxymethyl)aminomethane, EDTA.Na_2 and boric acid (10/1/0,75), as electrolyte. The nomination of hemoglobin phenotypes was achieved depending on the migrating speed of the electrophoretic bands *(Hrinca, 1988)* (Figure 7).

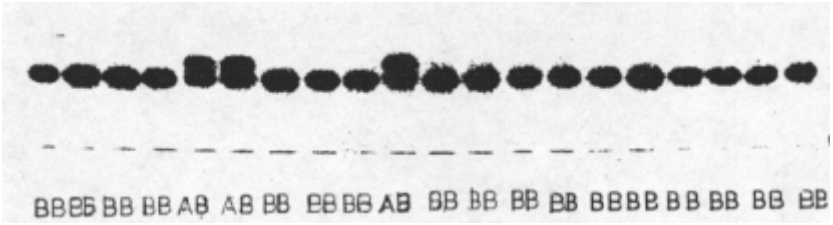

Figure 7. Haemoglobin electrophoregram in the Botosani Karakul sheep

The determination of potassium phenotypes was performed by flamphotometric method. The K^+ cationic concentrations of whole blood of the animals were expressed in mEq/l. Potassium phenotypes deceleration was made depending on the discontinuity space of the potassium ion distribution *(Hrinca et al, 2000)*.

The experimental results were analyzed statistically. The frequencies of the characteristics of qualitative traits of lamb pelts were calculated according to the

combinations of haemoglobin and potassium phenotypes (Hb/K). The test χ^2 was used for evaluating the significance of production differences among various phenotypic combinations Hb/K by the comparison method of empiric distributions *(observed frequencies)*.

Results and Discussion

The analysis of haemoglobin electrophoregrams revealed the existence of two haemoglobin patterns in the electrophoretic field in the Botosani Karakul lambs: phenotype HbA (with fast migration) and phenotype HbAB (with intermediate migration); Theoretically, it should also appear the phenotype HbA, but it is not expressed like in most sheep breeds *(Hrinca, 1988)*.

The flamphotometric determinations made on whole blood in the Botosani Karakul lambs have shown that the discontinuity of blood potassium distribution was recorded between 25 mEq/l and 30 mEq/l; the individuals with up to 25 mEq/l potassium blood are of phenotype LK (with low potassium concentration), and those with more than 30 mEq/l potassium blood are of phenotype HK (with high potassium concentration) *(Hrinca et al., 2000)*.

The associative analysis refers to the grouped distributional comparison of characteristics of the main qualitative traits found in the lamb pelts of Karakul type for each combination among all haemoglobin and potassium phenotypes.

Theoretically, in ovine species, there are six possible phenotypic combinations among the genetic variants of haemoglobin and potassium: HbAA/LK, HbAA/HK, HbAB/LK, HbAB/HK, HbBB/LK and HbBB/HK. In the Botosani Karakul breed, because of the absence of haemoglobin homotype HbAA, only four such combinations are expressed (HbAB/LK, HbAB/HK, HbBB/LK and HbBB/HK).

a) Association of phenotypic combination Hb/K with curl shapes (Figure 8). The curl shape is given by the special conformation and arrangement of hair fibres grouped as bundles in a certain sense and by the closure degree and curling direction of these fascicles.

The first four types of curls (“cylindrical tube”, “grain”, “cylindrical tube+grain” and “flat tube”) have been considered very valuable from economic point of view, due to the histochemical and morphological peculiarities of hair fibres as well as to the curling way of these fibres which creates a very nice and pleasant drawing on the surface of lamb pelt. Therefore, the superiority of one or other of these curls is a question of taste and fashion. If the first three curl shapes are considered traditional, the “flat tube” is a more recent phylogenetic appearance as a result of diversified practicing the selection criteria used for improvement of this breed. The fifth category of lambs have lamb pelts contain diverse types of curls (“varia”), irregularly arranged, creating a heterogeneous drawing that gives them low zooeconomic value.

The most valuable types of curls record the highest incidences in lambs with phenotypic combination HbAB/HK. The lowest frequencies of these types of curls are found in lambs in which the phenotype LK is associated with both haemoglobin phenotypes. Also, the lamb pelts of HbBB/HK association have an almost similar distribution of these curl types to those of the last two phenotypic combinations.

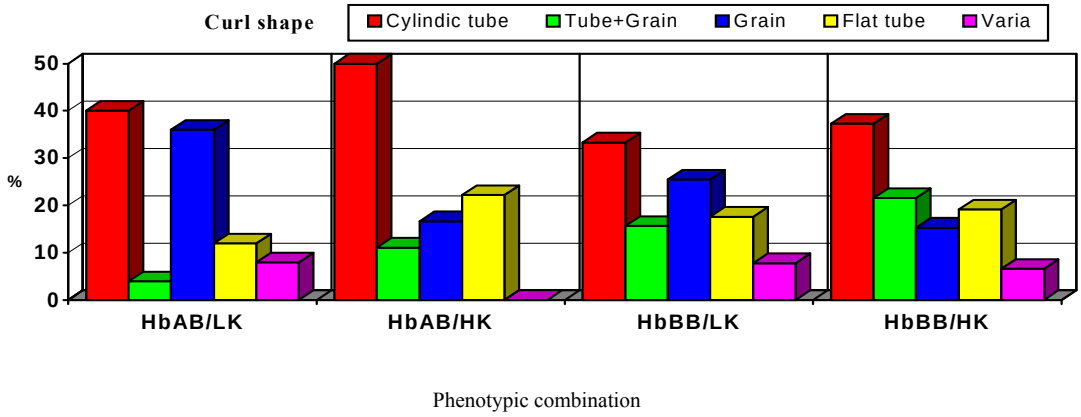

Figure 8. Incidences of curl shapes of the lamb pelts of Karakul type depending on Hb/K types

On the other hand, if the unvaluable curls of "varia" type are missing in the lambs HbAB/HK, they have an important share in the lambs with the other phenotypic combinations (especially those HbAB/LK and HbBB/LK). In fact, the lambs HbAB/HK possesses only valuable types of curls.

b) Association of phenotypic combination Hb/K with curl sizes (Figure 9). The curl size is determined by the length of component fibres, ratio between width and height of curls, diameters of these curls and their closure degree.

The first two sizes of curls ("middle" and "middle-small") present high economic value, while the curls with extreme sizes ("small" and "big") are undesirable traits in livestock practice of the Botosani Karakul sheep.

The valuable sizes of curls are much more frequently encountered in lambs with phenotypes HbAB and HK. Considerable frequencies of "middle" and "middle-small" curls are found in lambs HbAB/LK and HbBB/HK too. In the lambs of type HbBB/LK, these curls have the lowest distribution.

The "small" curls are the most frequently encountered in the lambs HbBB/LK. Lower frequencies, but taken into account, of these curls are in lambs HbAB/LK and HbBB/HK. The lowest incidence of "small" curls is registered in lambs HbAB/HK. The other unvaluable size of curls ("big") is less common in population, having a low frequency in lambs HbAB/LK and HbBB/HK, a sporadic spread in lambs HbBB/LK, while in the lambs HbAB/HK this feature is missing.

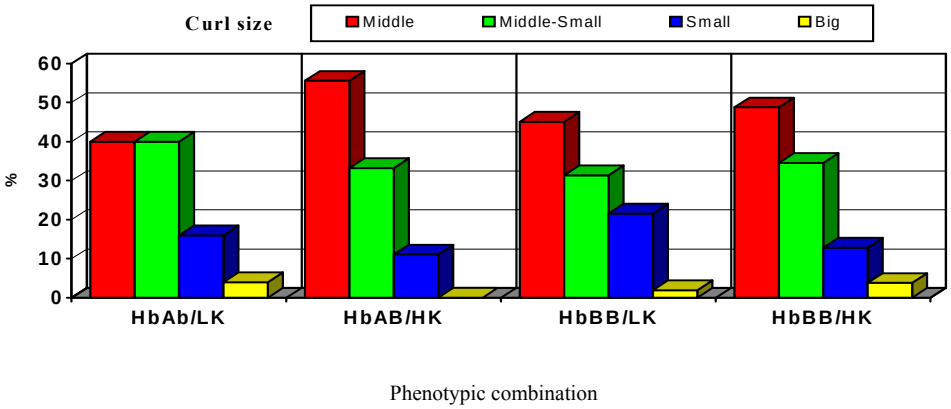

Figure 9. Incidences of curl sizes of the lamb pelts of Karakul type depending on Hb/K types

c) Association of phenotypic combination Hb/K with hair fibre quality (Figure 10). The quality of hair fibres is conditioned by the length, thickness, density, elasticity and uniformity of the component fibres of curls.

Of the four qualities of hair fibres, only the first characteristic ("silky") corresponds, in the highest degree, to the economic exigencies, and the second feature ("normal") is partially agreed in the selection activity, while the last two features ("rough" and "soft") must be eliminated from the lamb populations.

The hair fibres which confer the silken aspect of the lamb pelts are the most commonly found in lambs HbAB/HK. They are followed by the lambs HbBB/HK. In the phenotype LK associated with both haemoglobin phenotypes the silken fibres have a similar proportion. In the same lambs, one third of hair fibres have "normal" quality. In the lambs with phenotype HK associated with both haemoglobin phenotypes, the "normal" hair fibres have almost the same distributions.

The hair fibres with both unvaluable qualities ("rough" and "soft") are missing in lambs with phenotypic combination HbAB/HK. The rough fibre does not appear either in lambs HbAB/LK and HbBB/LK; the only phenotypic combination in which the rough fibres are present belongs to lambs HbBB/HK, but their presence is very low. Also, the soft fibres have a limited spread in phenotypic combinations HbBB/LK and HbAB/LK or very low in lambs of HbBB/HK type.

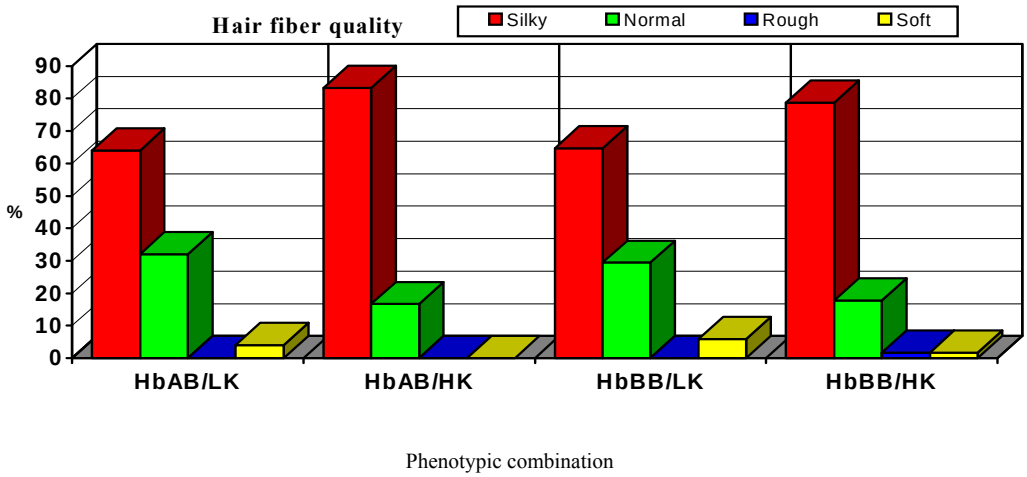

Figure 10. Incidences of hair quality of the lamb pelts of Karakul type depending on Hb/K types

d) Association of phenotypic combination Hb/K with hair fibre lustre (Figure 11).

The lustre of hair fibres expresses their ownership to reflect the light with a greater or lesser intensity. These feature depends on arrangement of fibres in curls, on surface structure of fibres and especially on the cuticle layer compactness and somehow of the cortical one of the hair fibres.

The "intense" lustre is one of the most important features of lamb pelts, but it is obtained enough difficult, its incidence being relatively low, so that the most valuable lamb pelts have a "good" lustre. The "satisfactory" lustre is more and more hardly agreed in the selection work, and the "mat" one gives a very weak quality to lamb pelts.

In the lambs HbBB/HK, one third of the lamb pelts have “intense” lustre and other two-thirds have "good" lustre. These two characteristics are very well represented also in lambs with the other three phenotypic combinations: HbAB/LK, HbBB/HK and HbBB/LK.

Both types of fibres with unvaluable lustre do not appear in lambs of HbAB/HK type. The “mate” fibres are missing in lambs of type LK associated with phenotypes HbAB and HbBB, and this fibre type is sporadically met in lambs with phenotypic combination HbBB/HK. The hair fibres with "satisfactory" lustre register low frequencies in lambs with phenotypic combinations HbAB/LK and HbBB/HK, and the incidence of this lustre type can be taken into consideration in the lambs HbBB/LK.

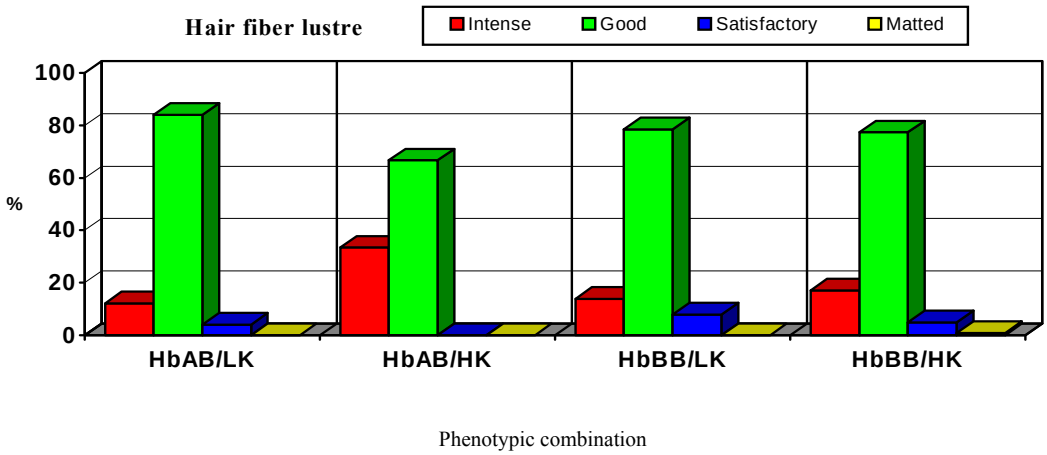

Figure 11. Incidences of hair lustre of the lamb pelts of Karakul type depending on Hb/K types

e) Association of phenotypic combination Hb/K with hair fibre colour (Figure 12). The natural colour of lamb pelts of Karakul type depends on the, intensity and distribution pattern of the pigment along the fibres, both on their surface and in their histological structure.

The most important qualitative feature of the Botosani Karakul sheep is the colour of the hair fibre of lamb pelts. Six colour varieties (lines) are meeting in the Botosani Karakul breed: black, greyish, brown, grey, pink and white. The colours black and greyish are traditional (classical), and the other colours give nobleness to this breed. Due to the genetic similitude among them, as well as to their low incidence within this breed, the brown, grey, pink and white varieties were grouped in subpopulation of coloured varieties (lines).

The black line is the best associated with phenotypic combination HbAB/HK. The phenotypic combinations HbAB/LK and HbBB/HK contain a little more than 50% individuals of black line, while the lambs HbBB/LK are somewhat less spread than half of their subpopulation. The best representation of the greyish colour is in the lambs of HbBB/LK type. Important frequencies of this colour line are registered in lambs HbBB/HK and HbAB/LK. The lowest incidence of this colour line is found in lambs HbAB/LK. The coloured lines are less common in the population. The most coloured individuals are with phenotype HbBB/LK. The incidences of coloured lambs with HbAB/LK and HbBB/HK are similar, while in the individuals HbAB/HK the frequencies of these colours are the lowest.

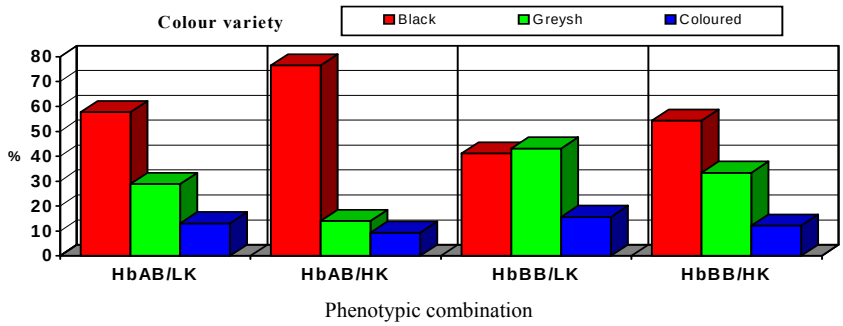

Figure 12. Incidences of hair colour of the lamb pelts of Karakul type depending on Hb/K types
*

From the morphological and histochemical point of view of the lamb pelts (shape and size of hair curls, quality and lustre of hair fibres), the most valuable features are associated with combination of phenotypes HbAB and HK, and the weakest association of these features occur with the phenotypic combination HbBB/LK. The phenotypes HbAB/LK and HbBB/HK present similar correlational aspects with the qualitative morphological and histochemical features of the lamb pelts.

From the colour point of view of the hair fibres, the colours which give nobleness to the Botosani Karakul breed (greyish and especially brown, grey, pink and white) are more associated with phenotypic combination HBB/LK, and the lowest of their spread is in phenotype HbAB associated with phenotype HK, but this phenotypic combination is associated to the highest degree with the black colour. The phenotypic combinations HbAB/LK and HbBB/HK resemble between them concerning their association with different colours of hair fibres.

So, the association of phenotypes HbAB and HK could contribute, to a great extent, to improving the qualitative morphological and histochemical features of lamb pelts, in exchange the phenotypic association between HbBB and LK seems to have a selective advantage on the other phenotypic combinations in the direction of the diversification and strengthening of the range of colours and of their shades.

The association of haemoglobin types with those of blood potassium should be attributed to the linkage between the loci Hb and K. Due to the existence of this linkage, some correlations can be established between different phenotypic combinations and the qualitative features of the lamb pelts by the influence of the potassium phenotype on the haemoglobin types, especially on the degree of affinity or dissociation of oxygen to the haemoglobin molecule. At the same time, the associative diversity of the phenotypic combinations Hb/K with the production traits would be due to the linkage of the loci Hb and K with the loci of other biochemical genetic systems, such would be glucose-6-phosphate, ceruloplasmin, glutathione, glucose-6-phosphate dehydrogenase or some blood group factors. But, probably, the main factor would be the metabolic profile of red cell in relation to its haemoglobin and potassium phenotypes dictated, it seems, to a great extent, by the phenomena of active cationic transport from the haematic wall level *(Yaman et al, 1986; Khan, 1987; Moradi Shahrbabak et al, 2007)*.

Comparing among them the empirical distributions of qualitative features of the lamb pelts in relation to all phenotypic associations of the haemoglobin and potassium systems, it comes out that among different subpopulations of lambs there are very great and very significant differences, concerning both each feature and all traits, the values of the test χ^2 exceeding the most critical threshold of significance (0.1%) (Tables 1 and 2).

Such studies have not been reported by the speciality literature. Similar studies refer to the association of qualitative features lamb pelts only with a simple biochemical genetic system, either haemoglobin *(Hrinca, 1988)* or potassium *(Hrinca et al., 2000)*. Correlational analysis of these two biochemical-genetic structures or of the combinations Hb/K with the sheep productivity had in view those with the quantitative production traits (meat, wool, milk) *(Lipecka et al., 1984; Yaman et al., 1986, Khan, 1997; Moradi Shahrbabak et al., 2007)*.

Table 1. Testing the differences between haemoglobin/potassium phenotypic combinations concerning the main qualitative features of lamb pelts of Karakul type

Production trait	Phenotypic combination	"t"	Liberty degrees
Curl shape	HbAB/LK – HbAB/HK	41.98***	4
	HbAB/LK – HbBB/LK	67.47***	
	HbAB/LK – HbBB/HK	99.08***	
	HbAB/HK – HbBB/LK	53.95***	
	HbAB/HK – HbBB/HK	70.01***	
	HbBB/LK – HbBB/HK	175.03***	
Curl size	HbAB/LK – HbAB/HK	41.91***	3
	HbAB/LK – HbBB/LK	67.22***	
	HbAB/LK – HbBB/HK	92.51***	
	HbAB/HK – HbBB/LK	53.55***	
	HbAB/HK – HbBB/HK	68.45***	
	HbBB/LK – HbBB/HK	170.25***	
Hair fibre quality	HbAB/LK – HbAB/HK	41.92***	3
	HbAB/LK – HbBB/LK	67.12***	
	HbAB/LK – HbBB/HK	105.34***	
	HbAB/HK – HbBB/LK	53.80***	
	HbAB/HK – HbBB/HK	68.23***	
	HbBB/LK – HbBB/HK	177.19***	
Hair fibre lustre	HbAB/LK – HbAB/HK	41.95***	3
	HbAB/LK – HbBB/LK	66.92***	
	HbAB/LK – HbBB/HK	92.48***	
	HbAB/HK – HbBB/LK	54.22***	
	HbAB/HK – HbBB/HK	70.66***	
	HbBB/LK – HbBB/HK	173.80***	
Hair fibre colour	HbAB/LK – HbAB/HK	41.96	2
	HbAB/LK – HbBB/LK	67.38	
	HbAB/LK – HbBB/HK	95.82	
	HbAB/HK – HbBB/LK	53.71	
	HbAB/HK – HbBB/HK	69.79	
	HbBB/LK – HbBB/HK	174.69	

Table 2. Testing the differences between haemoglobin/potassium phenotypic combinations concerning all qualitative features of lamb pelts of Karakul type

	Phenotypic combination	χ^2	Liberty degrees
All qualitative features of lamb pelts	HbAB/LK – HbAB/HK	41.94***	17
	HbAB/LK – HbBB/LK	67.22***	
	HbAB/LK – HbBB/HK	97.05***	
	HbAB/HK – HbBB/LK	53.88***	
	HbAB/HK – HbBB/HK	69.434***	
	HbBB/LK – HbBB/HK	174.19***	

Conclusion

In the Botosani Karakul sheep, the associations were established between the four combinations of the haemoglobin and potassium phenotypes existing in this breed, on the one hand, and the main qualitative features of lamb pelts, on the other hand.

The phenotypic combinations HbBB/KH is associated with the most valuable morpho-histochemical features of the lamb pelts (shape and size of curls, quality and lustre of hair fibres), while the phenotypic combination HbBB/LK is associated with the largest range of colours.

The differences among all phenotypic combinations Hb/K, in the terms of qualitative traits of lamb pelts, are very significant, having statistical assurance.

Povezanost fenotipskih kombinacija Hb/K sa osobinama kvaliteta krzna jagnjadi rase Botosani Karakul

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Rezime

Specifičnost proizvodnje ovaca koje pripadaju Botosani Karakul rasi je jagnjeće krzno karakteristično po različitim kvalitativnim osobinama (oblik i veličina pramenova, kvalitet, sjaj i boja vlakana pramenova), koje ovu rasu svrstavaju u plemenite i razlikuju je među drugim rasama. Ovo istraživanje je pokušaj asocijativne analize ovih osobina jagnjećeg krzna sa različitim kombinacijama između hemoglobinskih i kalijumskih fenotipova. Najvrednija osobina u pogledu oblika i veličine pramenova kao i kvaliteta i sjaja vlakana u pramenovima povezana je sa kombinacijom fenotipova HbAB i HK, dok je najslabija povezanost ovih osobina utvrđena kod fenotipske kombinacije

HbBB/LK. Sivkaste, braon, sive, roze i bele boje vlakana su više povezane sa fenotipskom kombinacijom HBB/LK, dok je najniža učestalost ovih boja utvrđena kod fenotipa HbAB uz fenotip HK, ali je ova poslednja fenotipska kombinacija u većem stepenu povezana sa pojavom crne boje. Razlike u empirijskim distribucijama kvalitativnih osobina jagnjećeg krzna u odnosu na sve fenotipske kombinacije hemoglobinskih i kalijumskih sistema su vrlo značajne, činjenica je kojom se preporučuje korišćenje ova dva biohemijsko-genetička sistema za unapređenje Botosani Karakul rase u proizvodnji jagnjećeg krzna.

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THE CONSEQUENCES OF USING MIXTURE WITH BUFFERS EFFECTS ON SOME BIOCHEMICAL PARAMETERS IN BLOOD SERUM DURING THE FATTENING OF THE LAMBS

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Original scientific paper

Abstract: This work has an aim to explore the effects of the product based on the natural zeolit during the fattening of the lambs and also the effects on contents of calcium, phosphorus and magnesium in blood serum. The experiment lasted 15 days in the closed area of the Agricultural school in Kraljevo, and the lambs were divided into three groups of 15 lambs (Control group –C, Experimental group E1 and E2). They were fed with sheep's milk, concentrate for lambs' fattening and meadow hay. The lambs in the experimental groups, in contrast to Control group, were fed with the different concentration of the preparation based on the natural zeolit (O_1=1%, O_2=1.5%), so that the manifested differences would be treated as the resault of the different concentration of the zeolit that was added to the lambs' food.At the end of the experiment the differences in the parameters of the lambs' blood serum were distinguished and especially in the concentration of calcium, phosphorus and magnesium.

Key words: lambs, zeolit, calcium, phosphrus , magnesium , blood serum

Introduction

The fodder that is covered with mould may be physically, chemically and energetically damaged that cannot be used as food *(Stojković et al., 1996; Radovanović et al., 1997)*. Considering that during the last few years it has been researched the possibility of using the natural zeolite in cattle breeding. Since natural zeolite is able to absorb different spoilt material in cattle organism (micotoxin, heavy metals, carbon monoxide, ammonia, radionuclide etc), thereby it can contribute to better production results and sustain health *(Masić et al., 2003, Adamović et al., 2001, Adamović et al., 2003)*. Many experiments showed that

products based on zeolite bind toxins from food and thereby it has a good influence on production results. Considering above mentioned facts, the subject of the experiment would be the perceiving of biochemical values of fattening lambs' blood serum (calcium, phosphor, magnesium) fed by preparation based on zeolite in different amount of added, under commercial name Mix Plus in order to find possible solutions for effective, qualitative and ecologically desirable production of lambs' meat.

Materials and Methods

The experiment is taken on the farm of the secondary agricultural school "Dr. Djordje Radic" in Kraljevo, on the lambs that are produced by crossbreeding of "pramenka" lambs and " vitemberg "rams. There were 45 lambs. The experiment is based on group control system. The lambs that were chosen had ear tags and they were divided into three groups of 15 lambs. There were made three compartments which were properly signed as K, O1 and O2 with 15 lambs in each. They could drink water as much as they want with the help of automatic drinkers. Microclimatic and zoo sanitary conditions were optimal and pretty the same for all three groups. The meals were adjusted to the lambs' age and were consisted of milk, fodder and hay and the meals were configured and technologically usual that is the meals are used in regular farm breeding. The only difference between the groups was in the examined product that was added to the meals, so the manifested differences were treated as the consequences of the different amount of zeolite added in food.In order to accomplish that we analyzed nutritive and chemical structure of the fodder with standard and regular methods. During the experiment the blood of the lambs were taken at the beginning and at the end of the experiment.

Results and Discussion

At the beginning of the experiment the amount of calcium in the lambs' blood was in normal amount, the average amount was between 2.20 and 2.30 m mol/l (Table 1a). The other authors came up with the similar results *(Jovanović, 1986.)*. We have to confirm that in period after decision the concentration of calcium, although physiologically normal amounts, was under average of 2.50m.mol/l. At the end of experiment the concentrations of calcium were normal, K1 = 2.55mmol/l, O1 = 2.55mmol/l and O2= 2.64mmol/l (Table1b). Analyzing the concentration of calcium in lambs' blood from O2 group we can say that it was the lowest at the beginning and the highest at the end of the experiment in comparison to other two groups. We can conclude that it is the result of positive influence of

increased amount of natural zeolite (1.5%) for this group on using mineral substances from food. We must say that manifested differences are not significant.

Table 1. Level of variation of Ca (mmol/l)

group	Level of variation of Ca (mmol/l) (a)				
	x	Sd	Sx	Cv	%
K	2.28	0.47	0.12	0.21	100.00
O1	2.30	0.51	0.13	0.22	101.01
O2	2.20	0.54	0.14	0.25	96.32

group	Level of variation of Ca (mmol/l) (b)				
	x	Sd	Sx	Cv	%
K	2.55	0.55	0.14	0.22	100.00
O1	2.55	0.57	0.15	0.22	99.96
O2	2.64	0.58	0.15	0.22	103.49

At the beginning of the experiment the concentration of phosphor in the lambs' blood was normal and average. It extended from 1.28mmol/l for K1 group up to 1.44mmol/l for O2 group which is in compliance with literature data (Table 2b). At the end of the experiment the increased amount of phosphor was noted but just in physiological boundaries and differences among the groups are insignificant (Table 2b). We have to emphasize that the lambs that were fed with Mix Plus based on natural zeolite had the increased concentration of phosphor at the end of the experiment , so we think that it has a positive influence on activity of micro flora and increased usage of phosphor.

At the beginning of the experiment the concentration of magnesium in the lambs' blood was equable and extended from 61.41mg/kg up to 61.75 mg/kg (Table 3). Our results match with *Jovanović et al. (1986)* results. We have to emphasize the fact that the lambs at the beginning of their lives, that is in the period of denial, have the amount of magnesium that are normal for grown animals.

At the end of the experiment we conclude that the concentration of magnesium is similar to the one at the beginning, which is confirmation of a very strong mechanism of saving the constant concentration Mg.

Table 2. Level of variation of P (mmol/l)

group	Table 2. Level of variation of P (mmol/l).(a)				
	x	Sd	Sx	Cv	%
K	1.28	0.32	0.08	0.25	100.00
O1	1.42	0.45	0.12	0.32	110.52
O2	1.44	0.51	0.13	0.35	112.08
group	Table 2. Level of variation of P (mmol/l).(b)				
	x	Sd	Sx	Cv	%
K	2.32	0.52	0.13	0.22	100.00
O1	2.40	0.41	0.11	0.17	103.27
O2	2.47	0.47	0.12	0.19	106.42

Table 3. Level of variation of Mg (мg/kg)

group	Level of variation of Mg (мg/kg). (a)				
	x	Sd	Sx	Cv	%
K	61.41	18.49	4.77	0.30	100.00
O1	61.75	17.94	4.63	0.29	100.55
O2	61.61	18.16	4.69	0.29	100.33
group	Level of variation of Mg (мg/kg). (b)				
	x	Sd	Sx	Cv	%
K	61.89	18.43	4.76	0.30	100.00
O1	61.48	17.20	4.44	0.28	99.34
O2	61.86	17.81	4.60	0.29	99.95

Conclusion

Based on the performed researches and results the following conclusions are made:

The concentration of Ca in the lambs' blood of O2 group was the lowest at the beginning and the highest at the end . It was 2.64mmol/l. We can conclude that it is the result of positive influence of increased amount of natural zeolite (1.5%) for this group on using mineral substances from food.

We have to emphasize that at the end of the experiment the lambs that were fed with Mix Plus based on natural zeolite had the increased concentration of phosphor, so we think that it has a positive influence on activity of micro flora and increased usage of phosphor.

At the end of the experiment we conclude that the concentration of magnesium is similar to the one at the beginning , which is confirmation of a very strong mechanism of saving the constant concentration of Mg. Adding the mixture with buffer activity did not influence on the amount of Mg in lambs' blood serum.

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Efekat korišćenja smeše sa pufernim dejstvom na neke biohemijske vrednosti krvnog seruma u tovu jagnjadi

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Rezime

Istraživanja u ovom radu postavljena su sa ciljem da se ispitaju efekti korišćenja preparata na bazi prirodnog zeolita u ishrani jagnjadi u tovu kao i utvrđivanje efekata na sadržaj kalcijuma, fosfora i magnezijuma u krvnom serumu. Ogled je izveden u trajanju od 90 dana, u zatvorenom objektu Poljoprivredne škole u Kraljevu, a eksperimentalne životinje su podeljene u tri grupe po 15 jagnjadi (Kontrolna-K i Ogledne-O1 i O2). Obrok se sastojao od ovčijeg mleka, koncentrata za tov jagnjadi i livadskog sena. Jagnjad oglednih grupa, za razliku od kontrolne, dobijala su različite koncentracije preparata na bazi prirodnog zeolita (O_1=1%, O_2=1.5%), kako bi se na taj način ispoljene razlike tretirale kao posledica sadržaja različite količine dodatog zeolita u hrani. Na kraju ogleda su utvrđene razlike u ispitivanim parametrima iz krvnog seruma jagnjadi i to u koncentraciji kalcijuma, fosfora i magnezijuma.

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THE INFLUENCE OF NUMBER OF LACTATION ON MILK YIELD PARAMETERS IN GERMAN FAWN GOATS

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Abstract: Goat breeding is one of the least developed agricultural sectors in Serbia. This is reflected in the fact that the total number of goats in Serbia is less than 200,000, as well as the fact that no goat milk production has been recorded by the statistics. Over the last decade, there has been great interest of breeders for intensive breeding of highly productive goats, which resulted in imports of breeding goats. In the same period, several herds of the Alpine breed and German fawn goats were formed, while their production results in the growing conditions in our country have not been fully studied yet. During the first three lactations in the period 2003-2009, the following parameters of milk yield were studied: length of lactation, milk yield in one lactation, percentage and the milk fat yield, and percentage and the total protein yield. The average lactation period was 247 days for the first, 260.6 days for the second and 266.8 days for the third lactation. The average of milk yielded was constantly increasing from the first (594.51 kg) towards the third lactation (761.96 kg), showing statistically highly significant differences (P<0,001). What was also observed is that the order of lactation has not significantly affected the percentage of proteins in milk, whereas the total protein yield in lactations was influenced by the order of lactation due to the differences in average milk yield in lactations.

Key words: German fawn goat, lactation, milk fat, protein, days of lactation, amount of milk

Introduction

The milk yield and composition of goat milk depends on the breed of goats, but is also heavily influenced by physiological and environmental factors. Thus, highly

productive breeds of goats in intensive production can produce up to 1,500 kg of milk in a single lactation, while in the same conditions some other goat breeds can produce a hundred of litres *(Krajinović, 2006)*. The most significant physiological factors are the following:

- Colostral period and stage of lactation. *Anifantakis and Kandarakis (1980)* state that the percentage of fat and protein is high in colostrum and in milk in the first phase of lactation, while it is significantly lower in the middle of lactation, to be increased again in the last stage of lactation while the quantity of produced milk is significantly reduced. The percentage of lactose remains within a rather narrow range during the whole period of lactation, whereas the concentration of minerals increases with the duration of lactation *(Mioč, 1991)*.
- Gravidity dramatically reduces milk yield according to *Salama et al. (2005)*, and without kidding, lactation can be prolonged for 2 to 4 years.
- Age of goats, i.e. number of lactation. It was found that milk production was the lowest in the first lactation, gradually increasing until the fourth, and sometimes until the sixth lactation, after which it declines.
- Litter size, i.e. the number of born kids is correlated with the amount of produced milk. Thus, goats with more kids per litter produce more milk than goats with single-born kids. This phenomenon is accounted for by mechanical stimuli on the udders of goats with more kids and by the impact of genetics. As stated by *Mioč and Pavić (2002)*, the impact of the litter size on milk yield is independent from the order of lactation, body weight of goats and the kidding season. According to the research conducted by a number of authors *(Subires et al., 1988; Crepaldi et al., 2000; Mioč, 1989)*, goats with more kids have longer lactation, and produce more milk and milk fat.
- Kidding season significantly affects the milk yield. Thus, *Crepaldi et al. (2000)* point out that goats kidded in the winter period (the interval from January to February) have higher milk production compared with the goats kidded in the spring (the interval from March to April) or in the summer (the interval from May to July).

Goat milk is considered to be the most complete and the most balanced food. Due to its exceptional quality, high nutritive value, easy digestibility and low allergenic potential, goat milk is recommended in the diets of children, adults and convalescents *(Ribeiro and Ribeiro, 2001)*. Nutritive and health aspects of goat milk are important for numerous medicinal problems of people allergic to cow's milk proteins. As stated by *Businco and Ballanti (1993)*, allergies to cow's milk are prevalent in human population, and this problem was diagnosed in 2.5% of children aged up to three year.

Due to the above stated facts, over the last decade farmers in Vojvodina have become interested in goat breeding, especially raising noble, highly yielding breeds. As there was the lack of such goats in our country, goats have been imported on several occasions; these were Alpine, Saanen and German fawn goats.

Since German fawn goats were not raised in our country before, there are no reliable data on production characteristics of this breed in our geographic and climatic conditions.

The aim of this paper is to supplement the research on milk yield traits of German fawn goats in geographic and climatic conditions of our country.

Materials and Methods

The research included German fawn goats from the reproductive herd of the goat farm "Select-milk" from Inđija, which is registered with the Department of Animal Husbandry, Faculty of Agriculture, Novi Sad, as a producer of high quality breeding livestock. During the period from 2003 to 2009, 269 first lactations, 178 second lactations and 97 third lactations of German fawn goats were analysed. The following milk yield parameters were studied: length of lactation, milk yield per lactation, the percentage and fat yield and the percentage and proteins yield.

The statistical parameters ($\bar{x}$, $S\bar{x}$, minimum, maximum) and t-test were calculated using the computer program Excel.

Results and Discussion

Lactation in goats starts with kidding, and finishes with drying of goats. In dairy goats, lactation can last virtually the whole year, i.e. from one until the following partus *(Krajinović, 2006)*. The total milk yield, milk fat and proteins depends on the duration of lactation, i.e. there is a positive correlation between the length of lactation and the total production of milk and milk fat *(Grossman et al., 1986)*.

Table 1. Duration of lactation of German fawn goats

	N	$\bar{x}$	$S\bar{x}$	Min - max
I lactation	269	246.95	1.40	120 - 292
II lactation	178	260.62	2.19	150 - 301
III lactation	97	266.77	3.39	150 - 309

On the basis of the results presented in Table 1, it can be observed that the average length of lactation, starting from the first lactation towards the third one, increased. The average length of the first lactation was almost 247 days, while the third lactation was almost 267 days. Statistical analysis determined that there is statistically highly significant difference ($P<0.01$) between the length of the first and the second lactation, and the first and the third lactation (at the level of $P < 0.01$), while there was no statistically significant difference between the second and

the third lactation. The obtained values of the lengths of lactation in German fawn goats are slightly higher than the values stated by *Ćinkulov et al., (2007)*. According to this author, the average length of lactation of German fawn goats is around 238 days. According to *Spatrh and Thume (2000)*, the average length of lactation for this breed is around 288 days.

The number of lactation markedly affects the milk yield. It was found that milk production is the lowest in the first lactation, to be gradually increased towards the fourth, and sometimes even to the sixed lactation, after which it declines *(Crepaldi et al., 2000)*.

Table 2. Milk yield (kg) at different lactations of German fawn goats

	N	$\bar{x}$	$S\bar{x}$	Min - max
I lactation	269	594.51	6.62	246 - 969
II lactation	178	678.93	12.30	272 - 1326
III lactation	97	761.96	19.47	252 – 1216

As expected, the average milk yield of the examined goats increased with every subsequent lactation. The average milk yield in the first lactation amounted to 594.51 kg, in the second lactation 678.93 kg, whereas the average milk yield in the third lactation of German fawn goat amounted to 761.96 kg. Statistical analysis showed that there are statistically highly significant differences ($P<0.01$) in the milk yield between all three observed pairs of lactations (between I and II, I and III, and II and III lactation). It can also be observed that there is statistically highly significant difference in the milk yield of the II and III lactation, while there was no statistically significant difference between the length of II and III lactation. What is also evident is that there is high variability of the milk yield within each lactation (even several times).

The most popular and widely used products from goat milk are cheeses. Production of cheese which will have consistent quality often includes many problems, the most significant of which is variable chemical composition of goat milk *(Morgan et al., 2001)*. One of the main parameters that influence the increase of cheese quantity and quality is its ability to coagulate and speed of coagulation. *Clark and Sherbon (2000)* found that goat milk with higher percentage of dry matter, protein and fat coagulates more quickly, forming firmer curd compared with milk with lower content of these components. Therefore, determining the parameters of milk yield traits is necessary in order to obtain information on the quality of milk for processing on one hand, and genetic potential of these parameters on the other hand.

Tables 3 and 4 present the average contents of milk fat and protein in different lactations of German fawn goats.

Nutrition of dairy goats directly affects the length of lactation, the amount of produced milk and its chemical properties. With regard to the chemical properties, the amount of milk fat is most affected by diet. Nutrition with larger quantities of bulky feeds has favourable effects on milk fat, while larger amounts of concentrate in meals result in increased milk yield, but also milk fat reduction. Variations are related not only to the total quantity of produced milk fat, but also to fatty acids composition. The quantity and quality of milk fat are directly dependent on the activity of stomach microorganisms and the amount of glucose in meals. Glucose with galactose in mammary complex forms lactose. Out of the total amount of glucose in body circulation, gland tissue of udders uses from 65% to 85% of it. The amount of lactose, proteins and minerals is less dependent on the diet *(Đorđević and Grubić, 2005)*.

Table 3. Milk fat content (kg and %) in milk of German fawn goats in different lactations

	N	$\bar{x}$		$S\bar{x}$		Min – max
		kg	%	kg	%	%
I lactation	269	18.37	3.11	0.23	0.017	2.30 – 4.44
II lactation	178	21.27	3.18	0.38	0.021	2.40 – 4.10
III lactation	97	24.22	3.24	0.59	0.029	2.65 – 4.06

Table 4. Protein content (kg and %) in milk of German fawn goats in different lactations

	N	$\bar{x}$		$S\bar{x}$		Min – max
		kg	%	kg	%	%
I lactation	269	18.12	3.06	0.20	0.013	2.80 – 3.79
II lactation	178	20.93	3.07	0.40	0.007	2.70 – 3.38
III lactation	97	23.40	3.07	0.57	0.008	2.88 – 3.30

Based on the results presented in Tables 3 and 4, it can be seen that the number of lactation affects milk fat content in milk of German fawn goats (in the first lactation it was 3.11%, while in the third lactation it amounted to 3.24%), whereas it has no significant effect on protein content in milk. Also, it can be noticed that milk fat content within every lactation is far more variable than protein content. By statistical analysis it was determined that there are statistically highly significant differences in milk fat content between I and III lactation ($P<0.01$), statistically significant differences between I and II lactation ($P=0.05$), while there are no statistically significant differences in milk fat content between II and III lactation. With regard to protein content, no statistically significant differences were determined between different lactations.

Conclusion

On the basis of the obtained results – the parameters of milk yield in the first three lactations of German fawn goats raised in the conditions of southern Vojvodina, the following conclusions can be reached:

- The number of lactation affects the length of lactation since the first lactation 246.95 days, the second lactation 260.62 days, and the third lactation 266.77 days.
- The number of lactation significantly ($P<0.01$) influences the milk yield during lactation, and in I lactation the milk yield was averagely 594.51 kg, to reach 761.96 kg in the third lactation.
- The number of lactation affects the content of milk fat in milk and it is statistically significantly higher ($P<0.01$) in goat milk in III lactation compared with I lactation, as well ($P<0.05$) as in II lactation compared with I lactation.
- No statistically significant differences were determined in average protein content in milk of the studied goats of different lactations.

Uticaj laktacije po redu na parametre mlečnosti nemačke šarene koze

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Rezime

Kozarstvo je jedna od najslabije razvijenih poljoprivrednih grana u Srbiji, što se ogleda kroz ukupan broj koza koji je manji od 200 000, kao i kroz činjenicu da statistika ne beleži proizvodnju kozjeg mleka. Tokom poslednje decenije zapaženo je veliko interesovanje odgajivača za gajenje visokoproduktivnih koza na intenzivan način, što je uslovilo uvoz priplodnih koza. U istom periodu je formirano nekoliko stada alpino rase i nemačke šarene koze, čiji proizvodni rezultati u uslovima gajenja u našoj zemlji nisu još uvek u potpunosti istraženi.

Tokom prve tri laktacije, u periodu od 2003. do 2009. godine, praćeni su parametri mlečnosti i to: trajanje laktacije, masa namuženog mleka po laktaciji, procenat i količina mlečne masti i procenat i količina proteina.

Prosečna dužina laktacije je iznosila: za prvu laktaciju 247 dana, za drugu laktaciju 260,6 dana, a za treću laktaciju 266,8 dana. Prosečna količina namuženog mleka se konstantno povećava od prve laktacije (594,51 kg) do treće laktacije (761,96 kg) i pokazuje statistički vrlo značajne razlike ($P<0,01$). Takođe je utvrđeno da redosled laktacije nije bitno uticao na procenat proteina u mleku, što se

ne može reći i za ukupne količine proteina u laktacijama obzirom na razlike u prosečnim mlečnostima laktacija.

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RESEARCH ON THE IMPROVEMENT OF THE MEAT PRODUCTION IN THE ROMANIAN TELEORMAN BLACK HEAD SHEEP BY CROSSING WITH MEAT BREEDS

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Abstract: In Romania the main direction of sheep breeding was until two decades ago, wool production, while the milk and meat productions were left in the background. After the 1990s, particularly after the accession of Romania to the European Union, sheep production redirected toward milk and meat production focusing on the identification of the most adequate techniques to increase these productions. One solution to restore this sector is to produce suckling lambs or fat lambs, competitive on the foreign markets. Within this context, the objective of our paper, part of a larger program, is to use industrial crosses to improve meat production in the local sheep breeds. We present here the results of crossing the local Teleorman Black Head (TBH) with imported meat rams: Suffolk and German Black Head (GBH). The studies monitored the performance of the (milk) hybrids F1 compared to the local breed lambs. 60 lambs assigned to three groups (20 lambs per group) were weaned at 2 months, when control slaughtering was used to determine: the slaughter house and commercial yield, proportion of carcass parts, proportion of butchery parts, meat to bone ratio for each part, carcass measurements, chemical composition of the meat, fatty acids and cholesterol included. At birth, the local breed lambs had 4.77 kg and a gain of 0.253 kg and reached 18.31 kg at slaughter. GBH hybrids lambs weighed 4.76 kg at birth, a gain of 0.277 kg and reached 20.63 kg at slaughter; Suffolk hybrids lambs weighed 5.36 kg at birth, 0.322 kg daily weight gain and 24.92 kg at 2 months. The slaughterhouse yield, the meat to bone ratio and carcass dimensions were better in the hybrids than in the local breed. The Suffolk and German Black Head rams transmitted to their progeny a higher speed of growth, a conformation specific to the meat breeds and a better dressing in muscles, a higher slaughter yield and a better meat to bone ration than the local breed lambs.

Key words: crossing, lamb meat production, local breed

Introduction

For the efficient production of sheep meat crossing is recommended as rearing strategy for the commercial farms. As reproduction practice, doesn't presume the random mating of the breeds; rather, crossing presumes the systematic use of breeds for the production of special progenies *(Freking, 2004)*. For instance, a crossing program with a terminal ram presumes the use of a male with superior traits which increases carcass quality of the commercial lambs. All the progeny from such crossing are marketed for meat *(Abdullah, 2008; Zgur, 2003)*. Crossing providers two advantages compared to the pure breed rearing: the heterosis effect and the complementarities of breed aptitudes. Heterosis, or the "hybrid vigour" is the superiority of the hybrid progeny compared to the average superiority of its parents. Complementarities refer to the fact that there are no perfect breeds and that each breed has strong points and weak points. In a systematic crossing program the breeds are mixed in order to balance the positive and negative aspects of each breed involved in crossing.

Our purpose is to improve the meat production of the local Teleorman Black head sheep by crossing with rams from the specialised meat breeds.

Given these aspects, the purpose of our experiment was to study the rearing performance and the carcass traits in F1 hybrid (suckling) lambs from cross of Teleorman Black head (TBH) ewes and Suffolk rams and with the German Black Head (GBH) rams. The performances of Suffolk sheep are impressive: ewes with bodyweight up to 80 kg and rams with bodyweight up to 120 kg. The breed is widespread in England and in many other countries around the world; it is a meat breed reputed for the fast growth speed and for the outstanding meat quality. Suffolk rams were used in many crossing designs for the production of meat hybrids *(Font Furnols, 2006; Leymaster, 1981, 1993)*.

The black head German meat sheep counts over 300,000 animals in Germany, 13,000 being registered into the herd-book. It is spread in central Germany. It's formation started in 1870 in northern Germany by a complex cross of the British short-hair meat breeds Hampshire, Oxfordshire, Shorpshire and Suffolk, followed by a vigorous selection. Today it is the second sheep breed in Germany in terms of population (17%). It achieves high performances, it is precocious but demanding, requiring cultivated pastures and free access to concentrate feeds. It resembles to its forming breeds, but it has dark brown hair on the head (compared to black in the Suffolk) and naked mandible and maxillary (short hairs in Hampshire and Oxfordshire). The disadvantages of this breed are the shorter body and the low milk production. The body is properly developed, height of 65 cm and body weight of 70-85 kg for the ewes and 75 cm and 110-135 kg for the rams. The growing lambs have a very high daily gain, 420-450 g and 60% slaughter yield. The sheep produce 4-5 kg wool annually with 30-35 microns finesses, 7-8 cm fleece length

and 48-50 washing yield. An important trait of these breed is its high prolificacy, 180-200%, the reproductive precocity (the young ewes enter reproduction at 10-12 months) and the very long mating season *(Pajor, 2004)*.

Materials and Methods

The research was conducted in the experimental farm of INCDBNA, where the Teleorman Black head sheep were assigned to three groups. The ewes from the first group were mated to a local ram, the ewes from the second group were mated to a Suffolk ram, while the ewes from the second group were mated to a German Black Head (GBH) ram. The studies monitored the growth performance and carcass characteristics in F1 hybrid nursing lambs compared to the local breed. Sixty lambs were assigned to three groups (20 lambs per group) and were weaned at the age of 2 months when control slaughtering was performed and the slaughterhouse and commercial yields were calculated; carcass measurements were performed and the meat to bone ratio was calculated, as well as the chemical composition of the meat, the fatty acids and cholesterol level, separately for each group. During the nursing period the lambs were weighed periodically, up to weaning. The carcasses were cut according to the French method which uses the following parts leg, loin, rack, shoulder, flank and neck.

Results and Discussion

Table 1 shows the weight gain of the experimental animals during the nursing period. Lamb weight at birth was higher in the hybrids with Suffolk, compared to the other breeds which had similar body weights. The differences between the weight at birth are significant between the hybrids with Suffolk and the local breed and between the hybrids with Suffolk and the German breed. At the age of one month, the differences persisted, but the local lambs scored a slight advantage over the GBH hybrids. At the age of two months, when they were slaughtered, the Suffolk hybrids had the highest weight, followed by the GBH hybrids, which were 2.32 kg heavier than the local breed. Significant differences were noticed only between the Suffolk hybrids and the local breed. The Suffolk hybrids had an average daily weight gain of 0.322±0.020 kg during the two experimental months, the GBH hybrids had an average daily weight gain of 0.277±0.022 kg, while the local lambs had an average daily weight gain of 0.253±0.015 kg. The Fisher test was used to test the significance of the differences between the average daily gains, which showed that the differences were significant.

Table 1. Weight gain of the lambs from birth to weaning (2 months)

Items	Teleorman Black head		F1 hybrids (Teleorman Black head x Suffolk)		F1 hybrids (Teleorman Black head x German Black head)	
	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%
Weight at birth, kg	4.770±0.139*	12.742	5.364±0.109*	7.617	4.760±0.175*	15.120
Weight at one month, kg	13.400±0.682	23.321	15.032±0.858	21.351	13.176±0.924	28.915
Weight at two months, kg	18.305±0.712*	17.819	24.921±1.400*	21.011	20.629±1.525	25.033
Weight gain, kg	13.532±0.687	23.244	19.558±1.104	26.877	15.869±1.163	30.205
Average daily weight gain, g/day	0.253±0.015	27.773	0.322±0.020	23.392	0.277±0.022	32.515

Fifteen lambs were slaughtered after weaning, five from each group. Table 2 shows the slaughtering results. The yield was higher in the Suffolk hybrids (49.027%) than in the GBH hybrids (48.761) and than the local breed lambs (47.212 %), the differences existing in the commercial yield too. The Fisher test showed that the differences were not significant.

Table 2. Live weight, slaughtering yield and proportion of the different carcass parts

Items		Teleorman Black head n=5		F1 hybrids (Teleorman Black head x Suffolk) n=5		F1 hybrids (Teleorman Black head x German Black head) n=5	
		$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%
Live weight. kg		21.42±0.928	9.694	25.5±0.375	2.917	21.720±0.487	5.016
Carcass weight. kg		10.14±0.653	14.420	12.7±0.040	0.642	10.180±0.449	9.881
Slaughtering yield %		47.212±1.381ns	6.544	49.027±0.747	3.000	48.671±1.806	8.295
Commercial yield %		51.752±1.364ns	5.897	54.455±0.833	3.048	52.920±1.804	7.621
Head	kg	0.899±0.061	14.992	1.010±0.048	9.667	0.877±0.013	3.411
	%	4.184±0.136	7.293	3.956±0.149	7.579	4.042±0.059	3.295
Organs	kg	0.976±0.069	15.928	1.220±0.072	11.892	0.922±0.023	5.669
	%	4.539±0.167	8.224	4.788±0.295	12.333	4.249±0.109	5.782
Full digestive tract	kg	4.891±0.285	13.030	5.050±0.268	10.616	5.013±0.542	24.182
	%	22.978±1.562	15.205	19.772±0.798	8.076	22.965±2.195	21.378
Hide	kg	2.556±0.130	11.378	2.801±0.165	11.797	2.283±0.164	16.131
	%	11.943±0.374	7.009	10.971±0.536	9.771	10.501±0.706	15.037
Legs	kg	0.572±0.034	13.149	0.688±0.014	4.177	0.587±0.015	5.764
	%	2.665±0.076	6.432	2.700±0.096	7.137	2.702±0.011	0.987
Losses	kg	1.3±0.202	33.706	2.021±0.079	7.860	2.075±0.145	15.708
	%	6.286±0.951	33.841	7.938±0.379	9.550	9.518±0.528	12.416

After slaughter the carcasses were left to dry for 24 hours, and then specific carcass measurements were performed: large trunk length, small trunk length, inner length of the leg, outer length of the leg, carcass width at the leg, thorax width, breast width, thorax depth, thorax perimeter, thigh perimeter. Table 3 shows that all length dimensions are larger at the local breed lambs than at the hybrid lambs, while the width dimensions (carcass width at the leg, thorax width, breast width) are larger in the hybrid lambs. The largest differences were noticed for the thigh perimeter: 56.25±0.750 cm in the Suffolk hybrid lambs and 52.8±0.860 cm in the GBH hybrids, compared to 35.5±3.49 cm in the local Teleorman Black Head lambs. The carcass dimensions of the GBH hybrids are slightly smaller than those of the Suffolk hybrids, but higher than those of the local breed.

Table 3. Specific carcass measurements (cm)

Items	Teleorman Black head		F1 hybrids (Teleorman Black head x Suffolk)		F1 hybrids (Teleorman Black head x German Black head)	
	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%
Large trunk length	66.4±1.122	3.780	62.75±0.946	3.016	62.4±1.666	4.179
Small trunk length	54.4±2.064	8.484	54.5±0.957	3.513	52.8±1.067	4.521
Inner length of the leg	27.2±1.392	11.450	21.25±0.478	4.505	22.2±0.374	3.768
Outer length of the leg	43.2±1.019	5.278	39.5±0.645	3.268	38.2±1.240	7.264
Carcass width at the leg	17.0±2.129	28.005	19.5±0.288	2.960	18.8±0.200	2.378
Thorax width	16.5±0.387	5.248	20.25±0.629	6.213	18.4±0.244	2.976
Breast width	14.4±0.244	3.803	17.0±0.408	4.802	15.8±0.663	9.387
Thorax depth	22.5±0.547	5.443	22.75±0.250	2.197	21.4±0.509	5.327
Thorax perimeter	58.2±1.199	4.610	63.0±0.816	2.592	59.0±1.414	5.359
Thigh perimeter	35.5±3.485	21.955	56.25±0.750	2.666	52.8±0.860	3.643

The differences noticed between the carcass dimensions of the three groups are due to the hybrid Suffolk and German Black Head rams specialised in meat production. The conformation of the meat breeds is brevimorphic and it is characterised by a stronger development of the width and depth of the animals, the body having the shape of a cylinder, with well developed muscles. These traits of the ram have been passed on to its progeny.

After measurements were performed, the carcasses were cut according to the French method which uses the following parts leg, loin, rack, shoulder, flank and neck (Table 4).

Table 4. Proportion of the butcher parts

Items		Teleorman Black head		F1 hybrids (Teleorman Black head x Suffolk)		F1 hybrids (Teleorman Black head x German Black head)	
		$\bar{x} \pm S_{\bar{X}}$	CV%	$\bar{x} \pm S_{\bar{X}}$	CV%	$\bar{x} \pm S_{\bar{X}}$	CV%
Leg	(kg)	2.742±0.207	16.922	2.493±0.019	1.557	1.716±0.060	7.911
	(%)	31.518±1.005	7.1359	37.94±0.530	2.797	34.09±0.558	3.664
Loin	(kg)	0.626±0.056	20.201	0.430±0.015	6.976	0.414±0.027	14.677
	(%)	7.155±0.205	6.426	6.96±0.412	11.856	8.18±0.253	6.931
Rack	(kg)	1.027±0.075	16.458	0.748±0.031	8.486	0.622±0.037	13.386
	(%)	11.848±0.503	9.499	11.59±0.367	6.332	12.30±0.263	4.795
Shoulder	(kg)	1.721±0.0818	10.626	1.042±0.043	8.262	0.898±0.021	5.349
	(%)	19.916±0.496	5.572	16.15±0.467	5.794	17.89±0.553	6.923
Flank	(kg)	1.753±0.175	22.387	1.233±0.055	9.013	0.919±0.082	19.958
	(%)	20.048±1.263	14.095	19.16±0.658	6.872	18.08±0.945	11.690
Neck	(kg)	0.830±0.066	18.006	0.555±0.045	16.241	0.477±0.026	12.460
	(%)	9.515±0.172	4.058	8.20±0.706	17.237	9.45±0.271	6.412

The Suffolk hybrids have the largest hock, followed by the GBH hybrids. The GBH hybrids have the largest proportions of rack and loin (premium parts), while the local lambs have the largest proportions of shoulder and flank (second quality parts).

Each butcher part was deboned and the proportion of meat and bone and the meat to bone ratio were calculated for the entire carcass. Tables 5 and 6 show the results.

Table 5 shows that the proportion of meat in the main region, leg, is larger in the Suffolk hybrids (77.08±0.516%), followed by the GBH hybrids (72.22±1.315); the same can be noticed for the rack and loin, where the meat percentage is higher in the hybrids than in the local breed. The secondary butcher parts, the shoulder and flank, have slightly higher meat percentages in the local breed than in the hybrids.

Table 5. Meat and bone proportion of the butcher parts

Breed			Teleorman Black head		F1 hybrids (Teleorman Black head x Suffolk)		F1 hybrids (Teleorman Black head x German Black head)	
			$\bar{x} \pm S_{\bar{X}}$	CV%	$\bar{x} \pm S_{\bar{X}}$	CV%	$\bar{x} \pm S_{\bar{X}}$	CV%
Leg	meat	(kg)	1.952±0.1510	17.301	1.893±0.038	4.073	1.242±0.064	11.533
		(%)	71.22±1.463	4.593	77.08±0.516	1.340	72.22±1.315	4.073
	bones	(kg)	0.790±0.072	20.606	0.563±0.012	4.444	0.474±0.011	5.501
		(%)	28.78±1.463	11.365	22.92±0.516	4.506	27.78±1.315	10.588
Loin	meat	(kg)	0.415±0.043	23.190	0.318±0.020	12.696	0.296±0.025	19.088
		(%)	65.96±1.773	6.012	70.44±1.048	2.977	71.13±1.618	5.088
	bones	(kg)	0.211±0.016	17.365	0.133±0.004	7.225	0.118±0.003	7.090
		(%)	34.04±1.773	11.651	29.56±1.048	7.093	28.87±1.618	12.537
Rack	meat	(kg)	0.553±0.034	13.810	0.443±0.018	8.379	0.367±0.030	18.769
		(%)	54.24±2.707	11.159	59.07±2.103	7.121	58.70±1.668	6.353
	bones	(kg)	0.474±0.054	25.615	0.308±0.021	14.206	0.255±0.010	8.985
		(%)	45.76±2.707	13.229	40.93±2.103	10.275	41.30±1.668	9.031
Shoulder	meat	(kg)	1.273±0.062	10.915	0.755±0.033	8.769	0.641±0.023	8.132
		(%)	73.97±0.850	2.571	72.18±0.928	2.573	71.31±1.077	3.379
	bones	(kg)	0.448±0.026	13.313	0.290±0.007	5.075	0.257±0.006	5.771
		(%)	26.03±0.850	7.306	27.82±0.928	6.677	28.69±1.077	8.398
Flank	meat	(kg)	1.359±0.161	26.482	0.955±0.051	10.823	0.691±0.072	23.376
		(%)	76.80±1.942	5.654	76.87±1.621	4.218	74.83±1.555	4.647
	bones	(kg)	0.394±0.020	11.610	0.285±0.012	8.474	0.228±0.016	16.159
		(%)	23.20±1.942	18.715	23.13±1.621	14.018	25.17±1.555	13.816
Neck	meat	(kg)	0.528±0.033	14.229	0.349±0.033	18.997	0.335±0.030	20.355
		(%)	64.05±1.737	6.066	65.67±1.074	3.271	69.86±3.168	10.141
	bones	(kg)	0.302±0.0362	26.788	0.181±0.012	13.953	0.142±0.013	21.214
		(%)	35.95±1.737	10.807	34.33±1.074	6.256	30.14±3.168	23.511

The meat to bone ration for the entire carcass is 2.19:1 in the local lambs, 2.42:1 in the GBH hybrids and 2.68:1 in the Suffolk hybrids (Table 6), which shows that the meat to bone ratio is better in the hybrid lambs.

Table 6. Meat to bone ratio

Items	Teleorman Black head		F1 hybrids (Teleorman Black head x Suffolk)		F1 hybrids (Teleorman Black head x German Black head)	
	$\bar{x} \pm S_{\bar{X}}$	CV%	$\bar{x} \pm S_{\bar{X}}$	CV%	$\bar{x} \pm S_{\bar{X}}$	CV%
Meat to bone ratio	2,193±0,132	13.14	2.683±0.063	4.713	2.427±0.169	15.570

No significant differences in meat quality (Table 7) were determined between the three groups after the chemical analyses of the meat were performed.

Table 7. Chemical composition of the meat

Specification	Teleorman Black head		F1 hybrids (Teleorman Black head x Suffolk)		F1 hybrids (Teleorman Black head x German Black head)	
	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%
DM (%) 65ºC	35.250±1.863	11.8223	39.830±0.834	4.190	35.476±2.708	17.068
DM(%) 103ºC	94.930±1.006	2.3701	91.213±2.121	4.691	94.412±0.422	0.999
(%) CP	49.570±4.0132	18.103	40.235±5.551	27.595	43.978±4.181	21.256
(%) EE	39.548±4.079	23.065	42.988±3.771	17.546	45.372±4.832	23.811
(%) Ash	2.788±0.181	14.519	2.055±0.140	13.628	2.636±0.212	12.012

All groups of animals had the same profile of fatty acids (table 8): Lauric acid, Miristic acid, Miristoleic acid, Palmitic acid, Palmitoleic acid, C17:0 , C17:1, Stearic acid, Oleic Trans acid, Linoleic acid, Linolenic acid, Conjugated Linoleic acid, Arachidonic acid. The meat from the hybrid lambs had a higher level of polyunsaturated fatty acids (Linoleic, Linolenic, Conjugated Linoleic, Arachidonic), which means that this meat is healthier due to the content of omega 3 and omega 6 fatty acids.

Table 8. Fatty acids and cholesterol profile in the lambs

Specification	Teleorman Black head		F1 hybrids (Teleorman Black head x Suffolk)		F1 hybrids (Teleorman Black head x German Black head)	
	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%	$\bar{x} \pm S_{\bar{X}}$	Cv%
Cholesterol g%g	0.204±0.151	104.74	0.137±0.012	17.800	0.212±0.023	24.503
Lauric g%g EE	1.120±0.170	21.46	0.320±0.103	72.033	0.324±0.064	44.492
Miristic g%g EE	9.445±1.155	17.29	9.733±0.231	5.310	8.952±0.562	14.034
Miristoleic g%g EE	1.335±0.315	33.36	0.968±0.063	14.529	0.962±0.063	14.690
Palmitic g%g EE	27.090±1.570	8.19	27.803±0.565	4.546	27.018±0.400	3.474
Palmitoleic g%g EE	3.520±0.370	14.86	2.708±0.145	11.954	2.650±0.271	22.861
C17:0 g%g EE	1.030±0.030	4.11	1.223±0.049	8.859	1.300±0.036	6.178
Decaheptenoic g%g EE	1.160±0.090	10.97	0.693±0.044	14.265	0.702±0.060	19.043
Stearic g%g EE	10.110±0.840	11.75	11.875±0.598	11.257	12.440±0.942	16.935
Oleic Trans g%g EE	1.275±0.015	1.66	1.168±0.471	90.188	1.664±0.408	54.840
Oleic Cis g%g EE	35.820±3.450	13.62	31.258±0.664	4.751	31.458±1.692	12.028
Linoleic g%g EE	3.975±0.445	15.83	4.493±0.283	14.097	4.596±0.196	9.542
Linolenic g%gEE	0.580±0.120	29.26	1.125±0.080	15.975	1.118±0.078	15.613
Conjugated Linoleic g%g EE	0.265±0.005	2.66	0.550±0.036	14.771	0.524±0.061	25.978
Arachidonic g%g EE	1.565±0.453	39.30	1.078±0.129	26.709	1.152±0.159	30.840
Other fatty acids g%g EE	2.830±1.020	50.97	2.363±0.280	26.469	2.486±0.567	51.013

Conclusion

The local lambs weighed 4.77 kg at birth, had a daily weight gain of 0.253 kg, reaching 18.31 kg at slaughter; the hybrids with CNG weighed 4.76 kg at birth, had a daily weight gain of 0.277 kg reaching 20.63 kg at slaughter; the hybrids with Suffolk weighed 5.36 kg at birth, had a daily weight gain of 0.322 kg and reached 24.92 kg body weight at two months. The average daily weight gain during the nursing period was higher in the hybrid lambs than in the local breed lambs.

The slaughtering yield, the commercial yield and the meat to bone ratio were also higher in the hybrid lambs compared to the local breed lambs.

The carcass measurements have shown that the width and depth of the carcasses were higher in the hybrid lambs than in the local lambs, while the lengths were higher in the local lambs.

This experiment proved that the Suffolk and German Black Head meat rams transmitted to their progeny a higher speed of growth and the specific conformation of the meat breeds, with a better dressing of muscles particularly in

the higher quality buthcer parts, the leg and rack, a better slaughter yield and a better meat to bone ration than the local Teleorman Black Head lambs.

Istraživanje o unapređenju proizvodnje mesa rumunske teleorman crnoglave ovce ukrštanjem sa mesnatim rasama

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Rezime

U Rumuniji, glavni pravac u ovčarstvu do pre dve decenije, proizvodnja vune, dok su proizvodnja mleka i mesa bile u pozadini. Nakon 1990-tih, a posebno nakon pridruživanja Rumunije Evropskoj Uniji, ovčarstvo se usmerilo na proizvodnju mleka i mesa, fokusirajući se na odredjivanje odgovarajućih tehnika za povećanje ovih proizvodnji. Jedno rešenje za obnovu ovog sektora je bila proizvodnja jagnjadi ili tov jagnjadi, koji će biti konkurentan na stranim tržištima. U ovom kontekstu, cilj našeg rada, koji predstavlja deo jednog većeg programa, jeste korišćenje industrijskog ukrštanja za unapređenje proizvodnje mesa lokalnih rasa ovaca. U radu su predstavljeni rezultati ukrštanja lokalne teleorman crnoglave ovce (TBH) sa uveženim ovnovima: safolk i nemačka crnoglava rasa (GBH). U studiji je praćena proizvodnja mleka kod hibrida F1 u poređenju sa lokalnim rasama. 60 jagnjadi, podeljeno u tri grupe (20 jagnjadi u grupi), su odbijeni u uzrastu od 2 meseca, i na kontrolnom klanju su odredjivani klanični i komercijalni randman, udeo delova trupa, udeo partija/mesarskih delova, odnos mesa i kostiju u svakom delu trupa, mere na trupu, hemijski sastav mesa, masne kiseline i holesterol. Jagnjad lokalne rase su na rodjenju imala telesnu masu 4.77 kg i prirast od 0.253 kg i dostigla su telesnu masu pred klanje od 18.31 kg. Melezi sa GBH su imali telesnu masu na rođenju od 4.76 kg, prirast od 0.277 kg i dostigla su telesnu masu pred klanje od 20.63 kg; melezi sa safolk rasom su na rodjenju imala telesnu masu od 5.36 kg, 0.322 kg dnevni prirast i masu pred klanje od 24.92 kg u uzrastu od 2 meseca.

Klanični randman, odnos mesa i kostiju i dimenzije/mere na trupu su bile bolje kod meleza nego kod lokalnih rasa. Ovnovi safolk rase, kao i neamački crnoglavi ovnovi su na svoje potomstvo preneli osobinu bržeg porasta, konformaciju koja je specifična za mesnate rase i bolji randman/ prekrivenost mišićima, veći klanični randman i bolji odnos meso/kosti nego lokalne rase.

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POSSIBILITIES FOR THE USE OF GOAT MEAT IN THE PRODUCTION OF TRADITIONAL SUCUK

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Abstract: Two variants of sucuk were made: one of beef meat and beef tail fat and another of goat meat and goat tail fat with meat/fat ratio of 75/25 and the same ingredients. After filling, the sausages were hung to dry in a traditional smoking house (without the possibility to control the temperature or humidity). Weight loss, pH, non-protein nitrogen content, basic chemical composition, instrumental colour measurement and sensory evaluation were done for both variants. Both variants had an almost identical weight loss (36.98 beef sucuk and 36.25 goat sucuk). Changes in pH value and non-protein nitrogen content had the same tendency and end values did not differ. The basic chemical composition at the end of production indicates that both variants were of very good quality. L* and b* values did not differ, but there was a significant difference in a* value (11.72 beef and 14.15 goat). In terms of appearance, texture and taste, assessors gave poorer grades to goat sucuk, but these grades do not indicate that the product is unacceptable (they were more than 5). It is possible to replace goat tail fat with beef fat to appease the specific flavour of the product and to make it more acceptable to consumers who may not be used to such flavour.

Key words: sucuk, goat meat, instrumental colour, sensory evaluation

Introduction

Fermented sausages have been produced for quite a long time and there are various types of these sausages. Fermented sausages are defined as ground meat mixed with salt and curing agents, stuffed into casings and subjected to a fermentation process in which microorganisms play a crucial role *(Lücke, 1994)*. Lactic acid occurs as a product of fermentation; it lowers the pH value of the product and helps to create the colour, taste and texture of the product *(Cenci-Goga et al., 2008)*. Fermentation is followed by ripening during which a number complex reactions take place, whose outcome is the degradation of proteins, lipids and

carbohydrates, as a result of the activity of endogenous muscle enzymes and microbial enzyme *(Spaziani et al., 2009)*.

Sucuk is a Turkish-style dry fermented sausage, very popular in Turkey and a number of Middle East countries, as well as Europe *(Ercoskun and Özkal, 2011)*. As for Serbia, it is traditionally produced in the western mountainous regions, primarily in small plants or village households in autumn and winter when weather conditions (temperature and relative humidity) are favourable. Small pieces of beef meat leftovers from dry beef (traditional dry meat product) production and tail fat are used. Meat/tail fat ratio is around 80:20; they are ground (about 4–5 mm) and mixed with salt and spices. The mixture is usually added 2.0–2.5 % of salt. Spices used include powdered black pepper, fresh garlic and red paprika, though local recipes may vary. The mixture is stuffed into beef small intestine and tied with rope. The product is then smoked and dried for over thirty days under local weather conditions. The sensory characteristics of sucuk are the following: very dark red colour, flavour and odour are of spicy fermented beef meat, with mild smoke aroma; the texture is very specific due to the addition of beef fat.

As for goat meat, there is great interest in kid meat and during the past decades, due to its high biological value, demand for this meat has increased even in countries with a high standard of living *(Žujović et al., 2009)*. Over the past few years in Serbia, interest for goat breeding has been increasing, especially in some regions *(Memiši et al., 2009)*. Goat meat has a specific and strong aroma, and the meat of older animals has poor structure. However, goat meat can be very successfully used in the fermented sausage processing *(Cosenza et al., 2003; Nassu et al., 2002)*.

The aim of this study was to investigate the possibility of using goat meat in sucuk processing and also to describe their chemical and sensory quality characteristics.

Materials and Methods

Two variants of sucuk were made in a small processing plant of the Institute for Animal Husbandry (Belgrade) in the period January–February 2010: of beef meat (about 8% fat) and beef tail fat (Variant B), and of goat meat (about 8% fat) and goat tail fat (Variant G) in the ratio of 75/25. Both variants were added the same amounts of the following ingredients: 2.3% curing salt (NaCl with 0.6% of $NaNO_2$), 0.35% sucrose and 0.5% spice mixture (powdered black pepper, garlic and red paprika). Meat and tail fat were first frozen to –4 ^{o}C and then minced in a meat grinder (Seydelman 114, Germany) to about 1 cm. Cutting and mixing with ingredients was carried out in the cutter (Seydelman K60, Germany) to 5 mm particle size. The mixture was filled in beef small intestine of 38 mm. Sausages

were tied with rope and made into horseshoe shape. After stuffing, the sausages were hung to dry in a traditional smoking house (without the possibility to control the temperature or humidity), with the parameters varying between 10–15 ^{o}C and relative humidity (RH) 75–90%. They were then occasionally smoked.

Three sausages were taken from each group for all analyses. Sampling was carried out on production days 0, 1, 3, 7, 14, 21 and 30 to determine pH value, non-protein nitrogen (NPN) and weight loss. Chemical analysis was conducted at the beginning (day 0) and end (day 30) of production. Sausage colour was determined at the end of production, and sensory evaluation was carried out.

Six individual sausages were weighed on the scales (Chyo MK-2000B), with a 0.1 g precision ratio, in order to determine weight loss.

pH value was measured by pH-meter Hanna, HI 83141 (Hanna Instruments USA).

NPN content (mg/100g dry matter (DM)) was determined according to the method of *Hughes et al. (2002)*.

The chemical composition of meat was determined in the following manner: moisture by drying samples at 105 °C (ISO 1442, 1997); protein content by Kjeldahl method and multiplying by factor 6.25 (ISO 937, 1978); fat content by Soxhlet method (ISO 1443, 1973), and ash content by sample mineralization at 550–600 °C (ISO 936, 1998).

Sausage colour was determined by Chromameter CR-400 (Minolta Co. Ltd, Tokyo, Japan), in line with the CIE L*a*b* system (L* – lightness, a* – red colour share, b* – yellow colour share). The colour of the cross section at three fresh cuts was measured.

The evaluation of sensory characteristics of sausages was conducted by nine assessors with previous experience in the evaluation of dried fermented sausages. A numeric-descriptive scale with nine-point system was used to evaluate of appearance, cut appearance, colour, odour, texture and taste of sausages (1 – extremely unacceptable, 9 – extremely acceptable).

The results were processed by single-factor analysis of variance (ANOVA). The differences between individual averages were tested using Tukey's method. Significant differences were considered for $P<0.05$. Calculations were done with software Statistica 6.0 PL, for Windows (Statsoft inc.)

Results and Discussion

Weight loss was almost identical in both variants (Figure 1) and no statistically significant difference was observed. More intensive weight loss was recorded in both variants between days 7 and 14, and especially after day 14, when the achieved pH value was close to pI (Figure 2), which accelerates drying.

Initial pH values differ, the lower being in B variant, which is not unusual. Similar results were reported by *Soyer et al. (2005)* and *Soyer and Ertas (2007)* for beef

sucuk and *Nassu et al. (2002)* for goat fermented sausages. Intensive pH drop started on day 3 in both variants, because of production conditions resembling traditional ones. The drop of pH value in traditional dry sausages is slight and fermentation is long because of low temperatures. pH value close to pI was achieved around day 14, corresponding to weight loss which is more intensive from this day onwards.

pH value at the end of production was alike and stood at 5.13 (B) and 5.21 (G). *Bozkurt and Bayram (2006)* cited Turkish Standard Institute (TSI) that pH value of sucuk of good quality should be in the range of 4.7–5.2.

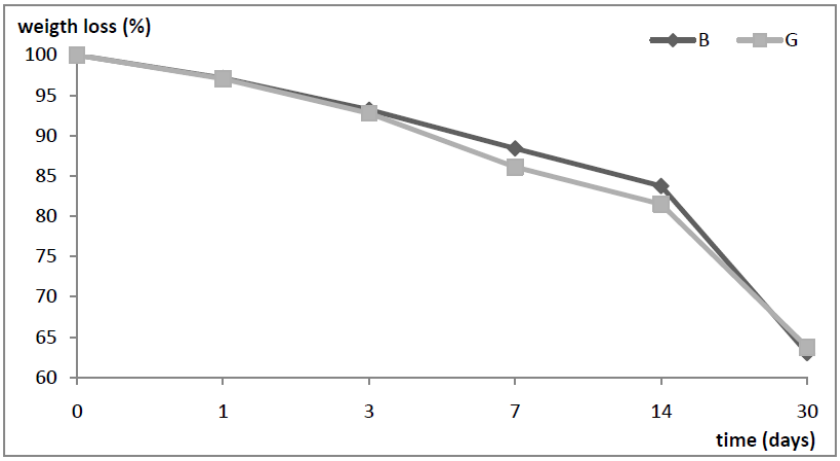

Figure 1. Weight loss of sucuks

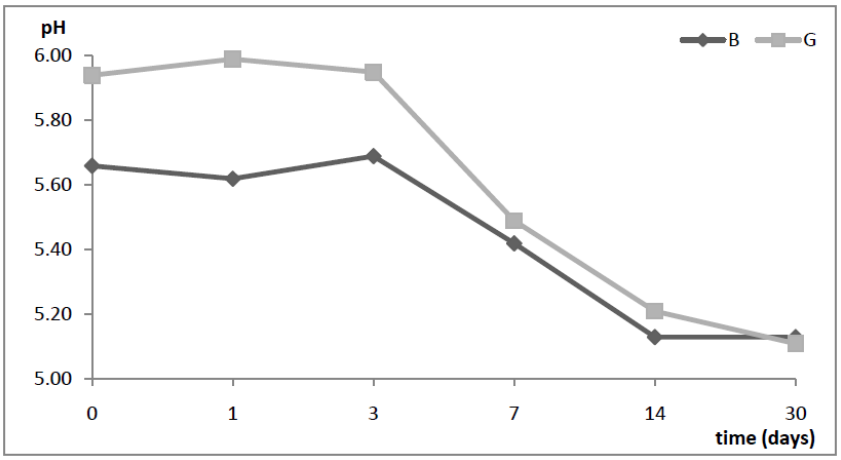

Figure 2. pH value changes during production

Table 1. Chemical composition of sucuk (%)

		Moisture	Fat	Ash	Proteins
Day 0	B	56.06±0.77	24.45±0.50	3.07±0.12	16.21±0.53
	G	57.39±0.78	23.19±0.37	3.02±0.02	16.16±0.82
Day 30	B	32.39±0.26	36.90±0.95	5.03±0.15	24.19±1.43
	G	33.03±0.12	36.61±0.63	5.09±0.14	25.10±0.61

The chemical composition of sucuk (Table 1) at the start of production was very similar and indicated careful selection of raw material in order to get balanced product content. The chemical composition at the end of production did not differ. Both variants of sucuk meet quality requirements envisaged by national regulations and contain less than 35% of moisture and more than 16% of meat proteins.

Changes in the NPN content (Figure 3) indicate protein degradation and the generation of low molecular weight peptides and free amino acids; they affect the generation of compounds, which in turn affect the pH value, taste and flavour in dry and semi-dry sausages *(Martín-Sánchez et al., 2011)*. The pattern of changes in the NPN content correspond to drop of pH value and increase more intensively after day 3. The pH value decline activates muscle proteinases, and also indicates the development of microflora, whose activity further affect proteolysis *(Spaziani et al., 2009)*. At the end of production, an increase of 30.76 % (B) and 41.23 (G) were recorded, in agreement with authors cited by *Fanco et al. (2002)*, who reported a rise of 50% and less.

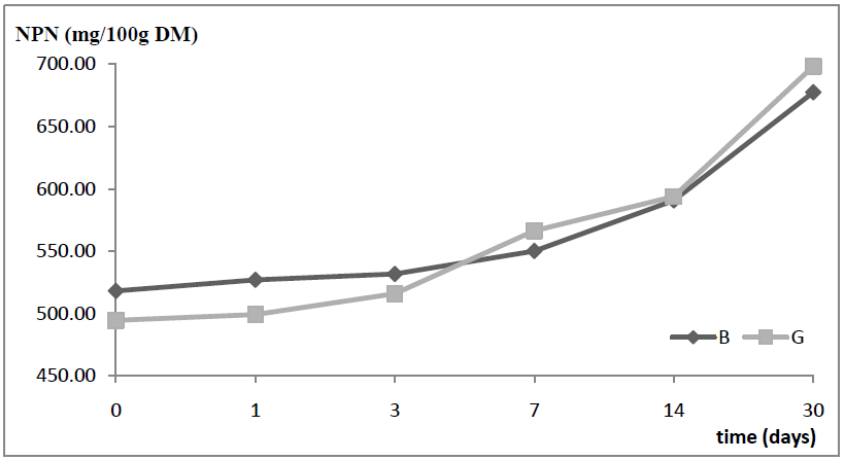

Figure 3. NPN changes during production

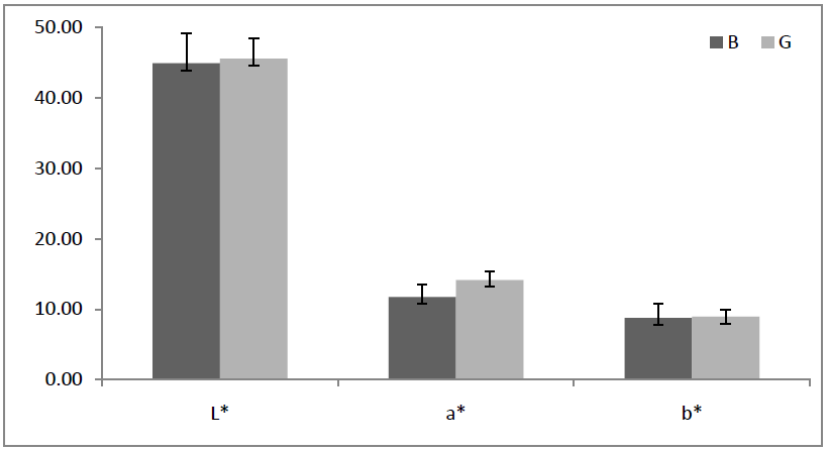

Figure 4. Colour evaluation

Insrumental colour measurement showed that there were no differences in L* and b* values, but that a* values statistically differed (Figure 4). Lower a* value in variant G (11.72) indicates that it was less bright red compared with goat meat variants (14.15). *Uren and Babayidit (1997)* reported for sausages obtained from various meat plants in Turkey and found that L* values were between 35.87 and 45.92, redness between 6.87 and 14.14 and and yellownes between 10.04 and 17.62.

Assessors did not find any statistically relevant differences between these two variants in terms of cut appearance, colour and odour (Figure 5). On the other hand, G sucuk received poorer grades in terms of appearance, texture and taste. Texture and taste are parameters that received the lowest grades in variant G (5.29 and 5.43), but these grades do not indicate that the product is unacceptable, as they are in the upper half of the grading scale (1–9). As goat meat and especially fatty tissue have specific taste, it is possible that they affected the lower grades. Acceptable taste can be achieved by substituting goat fatty tisue with that of beef, which, being more common, is more acceptable for the majority of consumers.

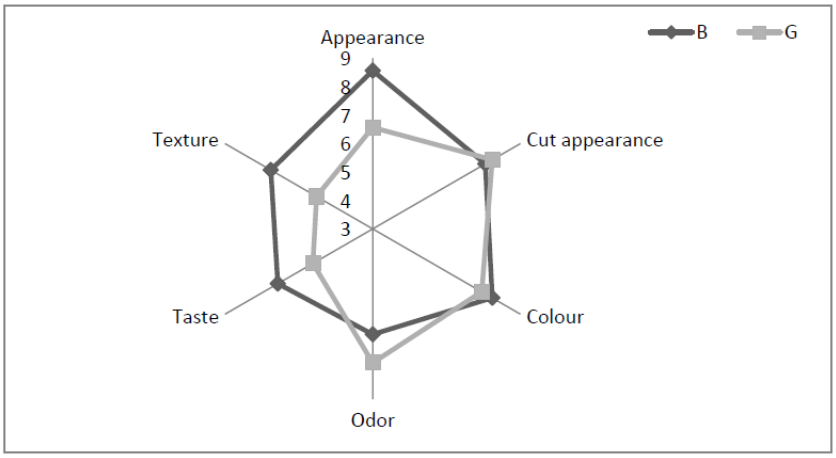

Figure 5. Sensory evaluation of sucuk

Conclusion

Research has shown that goat meat with goat fatty tissue may be used for the produciton of sucuk. There are no significant differences in terms of observed physical-chemical aprameters between the two variants at the end of production. Greater lightness (L*) indicates that the goat variant is more bright red compared with the beef variant, but sensory evaluation does not point to any significant difference in terms of colour. Because of the specific flavour of goat meat and fatty tissue, this variant of sucuk received poorer sensory evaluation grades in appearance, texture and taste. It is possible to replace sheep and goat tail fat with beef fat to appease the specific flavour of the product and to make it more acceptable to consumers who may not be used to such flavour.

Mogućnost upotrebe kozjeg mesa u proizvodnji tradicionalnog sudžuka

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Rezime

U Srbiji, sudžuk se tradicionalno proizvodi u planinskom delu, uglavnom u malim pogonima ili domaćinstvima, u jesenjem i zimskom periodu, kada to klimatski uslovi (temperatura i relativna vlažnost) dozvoljavaju.

U ovom ogledu napravljene su dve varijante sudžuka: prva od goveđeg mesa i masnog tkiva sa repa goveda i druga od kozjeg mesa i masnog tkiva sa repa koza. Kod obe grupe kobasica je odnos meso/masno tkivo bio 75/25 i korišćeni su isti začini i aditivi. Nakon punjenja, kobasice su sušene u tradicionalnoj pušnici. Kod obe varijante kobasica određen je: gubitak mase tokom sušenja, pH, neproteinski azot, osnovni hemijski sastav i izvršeno je instrumentalno merenje boje i senzorska ocena.

Obe grupe kobasice su imale skoro identičan gubitak mase tokom sušenja (36,98 kod goveđeg i 36,25 kod kozjeg sudžuka). Promene pH vrednosti i neproteinskog azota tokom zrenja su imale istu tendenciju i njihove krajnje vrednosti se nisu značajno razlikovale. Na osnovu podataka za osnovni hemijski sastav na kraju procesa proizvodnje, zaključeno je da su obe grupe kobasica bile veoma dobrog kvaliteta. L* i b* vrednosti se nisu značajno razlikovale, dok je statistički značajna razlika između grupa utvrđena za vrednost a* (11,72 kod goveđih i 14,15 kod kozjih kobasica). U pogledu senzornih karakteristika: spoljnog izgleda, teksture i ukusa, kozji sudžuk je ocenjen nešto slabije, međutim, takav proizvod je i dalje bio veoma prihvatljiv (sve ocene su bile iznad 5).

Jedan od načina da se smanji specifična aroma sudžuka napravljenog od kozjeg mesa, što bi ga učinilo prihvatlivijim za širu grupu potrošača, jeste zamena kozjeg loja sa goveđim.

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HELMINTHOSES OF GOATS BREEDING AT BELGRADE AREA

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Abstract: In aim of control parasitic infection of goat we started to sistematic parasitological examination of it. At Belgrade area we examined flocks of goats originated from 23 vilages from city districts Mladenovac, Lazarevac, Obrenovac, Grocka, Zemun, Surčin, Palilula, Voždovac and Zvezdara. Using standart coprological methods we examined 221 faecal samples. At same time, with necropsy we examined 67 animals. We found the following helminthes species: *Dicrocoelium dendriticum* (83,25%), *Moniezia expansa* (52,36%), *Echinococcus granulosus* (cyst) (83,25%), *Trchostrongylus axei* (79,82%), *T.colubriformis* (69,57%), *T. capricola* (62,85%), *Ostertagia circumcincta* (65,23%), *O. ostertagi* (23,33%), *Nematodirus spathiger* (83,25%), *N. filicolis* (43,31%), *Hameonchus contortus* (58,95%), *Skrjabinema caprae* (13,28%), *Chabertia ovina* (44,14%), *Oesophagostomum venulosum* (28,39%), and *Dictyocaulus filaria* (56,45%)

Key words: goats, helminthes, Belgrade

Introduction

Way of breeding usualy at shepeng had prerequisite to a lot of infections including parasitoses *(Quesada et al., 1990; Ashraf and Nepote 1989; Rattray, 2003)*. Pasture breeding make possible contact within sheep and eggs, larvar stages and intermediate host of *parasites (Fake,1990; Barger 1994; Pavlović et al.1995)*. Those induce that there are no one small ruminants without parasites *(Smith,1990; Jovanović et al.1991; Vujić et al.1991)*. Negative influence of parasitic infection reflected througt lost of weight and decrement quantum of lactation *(Mishra,1991; Luginbuhl,1998; Kristmundsson and Richter, 2000)*.

No study had previously made to determine endoparasites fauna of goats in Serbia. Ever, examination of goats parasitoses were only sporadically performed and we had only afew paper about it *(Vujić and Bošković,1981; Ilić,1990; Vujić et al.1991; Ilić et al.1991; Pavlović et al.1995)*.

From these reasoni in mind, we started with examination of parasitic fauna of goats at various pats of Serbia. At first, we started at Belgrade area, during 2003, and at our paper we presented results obtained in period 2009-2010.

Materials and Methods

During our examination we examined flocks of goats originated from 23 vilages from city districts Mladenovac, Lazarevac, Obrenovac, Grocka, Zemun, Surčin, Palilula, Vozdovac and Zvezdara. Using standart coprological methods we examined 221 faecal samples *(Pavlović and Anđelić-Buzadžić, 2010b)*. A total of 21 goats and lambs were examined in the slaughter house at Vojka. After slaughter we collected trachea, lung, heart, complete gastrointestinal tract, liver, kidney and urinary bladder and examined it at laboratory of Scientific Veterinary Institute. The intestine and the other organs were slit opened and visible helminths removed. Intestine contents were washing out and washing were scanned over a sieve, mesh aperture 150 μm, under jet water and the retained material examined small quantities at a time. Found parasites either fixing in 10% formalin,were mounted in lactofenol for identification, and mounted in Canada balsam. The determination was done by keys given by *Dunn (1978)*.

Results and Discussion

During examination we recorded 1 trematoda species, 2 cestoda species (one adult and one cystic form) and 12 species of nematode. Poliparasitismus and infection with gastointestinal helmints were established at all examined animals. Numerous parasites were found at small intestine, followed by abomasus and at colon.

We found the following helminthes species: *Dicrocoelium dendriticum* (83,25%), *Moniezia expansa* (52,36%), *Echinococcus granulosus* (cyst) (83,25%), *Trchostrongylus axei* (79,82%), *T. colubriformis* (69,57%), *T. capricola* (62,85%), *Ostertagia circumcincta* (65,23%), *O. ostertagi* (23,33%), *Nematodirus spathiger* (83,25%), *N. filicolis* (43,31%), *Hameonchus contortus* (58,95%), *Skrjabinema caprae* (13,28%), *Chabertia ovina* (44,14%), *Oesophagostomum venulosum* (28,39%), and *Dictyocaulus filaria* (56,45%)

Most prevalence species of nematode are *Trichostrongylus* and *Ostertagia* species. Although most of the gastro-intestinal species appear to follow this general pattern of seasonal distribution, some variations in intensively and duration of these characteristics with different worm species occurred. Thus with *Trichostrongylus* and *Ostertagia* species infection at mature goats the spring peak was more pronounced that the autumn infection *(Pavlović, 2009c,d; Pavlović et al., 2009b; 2010a,b)*. Prevalence of *Moniezia expansa, Dicrocoelium dendriticum* and *Dictyocaulus filaria* were simillar at other aprt of Serbia where we examined parasites of small ruminants *(Pavlović et al., 2003, 2008, 2010a)*.

Generally speaking the goat's parasites represent a global problem. The countries of Magreb (Morocco, Tunisia and Algeria), Middle East and Africa are in permanent battle with parasitic infections and losses ensued by them *(Quesada et al., 1990, Fakae, 1990, Wamae and Ihiga, 1990)*. Same situation are at Mediterranean basin where especially problem present *Echinococcosis/Hydatidosis* which occurred on goats and sheep at rate of 70-90% *(Pavlović and Ivanović, 2006; Pavlović et al, 2011a)*. Goat helnthoses had a worldwide distribution. We had a reports from Island *(Kristmundsson and Richter, 2000)* to NewZeland *(Rattray, 2003)*, from Pakistan *(Bilqees, 1988)* and India *(Mishra, 1991)*, to USA *(Ashraf and Nepote, 1990)*.

The data on harmful before and effect of parasitic infections on the goat performance undoubtedly show that in the anthelminth conditions of rearing high performance animals it is necessary to conduct the measures of prophylactic treatment *(Barger et al., 1994; Zajac and Gipson, 2000; Chartier et al., 2000; Kaplan et al. 2004; Shaik et al. 2004; Pavlović et al., 2009a)*.

Conclusion

However, since the parasitic infections are in majority sub clinical. The attention has not been paid to this problem in Serbia. The prophylactic treatment is not conducted in the majority of flocks or it is only partially performed what can be seen by the records from the slaughter line and from production results. In aim of introducing parasites fauna of goats and prepare measure to its control we must to continue our examination. This would be the only way to obtain better product results, characteristics and quality of goats and lambs meat in ecological breeding condition

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Helmintoze koza sa područja Beograda

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Rezime

U cilju kontrole parazitskih infekcija koza potrebno je utvrditi vrste i distribuciju parazita. U radu dajemo prikaz helminata koza sa područja Beograda. Istraživanja su vršena u 23 sela na području opština Mladenovac, Lazarevac,

Obrenovac, Grocka, Zemun, Surčin, Palilula, Voždovac i Zvezdara. Primenom standardnih koproloških metoda pregledan je 221 uzorak izmeta. Isto tako, ispitali za obdukciju 67 životinja. Ustanovljene su sledeće vrste helminata: *Dicrocoelium dendriticum* (83,25%), *Moniezia expansa* (52,36%), *Echinococcus granulosus* (cyst) (83,25%), *Trchostrongylus axei* (79,82%), *T. colubriformis* (69,57%), *T. capricola* (62,85%), *Ostertagia circumcincta* (65,23%), *O. ostertagi* (23,33%), *Nematodirus spathiger* (83,25%), *N. filicolis* (43,31%), *Hameonchus contortus* (58,95%), *Skrjabinema caprae* (13,28%), *Chabertia ovina* (44,14%), *Oesophagostomum venulosum* (28,39%), i *Dictyocaulus filaria* (56,45%)

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LEAF AND STEM CHEMICAL COMPOSITION OF DIVERGENT ALFALFA CULTIVARS

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Original scientific paper

Abstract: Alfalfa is a perennial crop that provides a higher yield of proteins per unit area than any field crop, which makes it highly desirable for the production of hay and feed for domestic animals (especially ruminants). The objective of this study was to determine the dry matter chemical composition in the whole plant, leaves and stems of five divergent alfalfa cultivars. Variations were found in the contents of proteins, hemicellulose, NDF, ADF, ADL, cellulose, and minerals depending on genotype and plant part. Significant differences existed between the cultivars in quality parameters, indicating that breeding for high alfalfa quality is justifiable.

Key words: alfalfa, proteins, fibers, minerals, cultivar, leaves, stems.

Introduction

Alfalfa (*Medicago sativa* L.) is a major forage legume worldwide. In Serbia, it is in the third place according to acreage (200.000 ha), after corn and small grains. Alfalfa produces more protein per hectare than grain or oilseed crops; therefore, it is widely used for hay production and as pasture for livestock *(Monteros and Bouton, 2009)*. Alfalfa is a highly valued animal feed. It is a rich source of proteins, fibers, minerals and vitamins used in the diet of livestock, especially ruminants. Alfalfa forage quality is determined by two main components: protein digestibility and protein content *(Hill et al., 1988)*. Digestibility of alfalfa organic matter depends on the contents of cellulose and lignin. As lignin is virtually indigestible, intensive lignification of cell wall in late stages of alfalfa development tends to reduce the coefficient of digestibility.

Protein content in alfalfa dry matter varies from18 to 25% depending on the growth stage, cultivar and storage method *(Katic et al., 2006)*. Alfalfa nutritive value is identified with protein content which depends on the share of leaves in dry matter yield which in its turn is positively correlated with protein content *(Julier et al., 2001; Katic et al., 2005)*. The proportion of leaves and stems in alfalfa hay can

vary greatly, depending on maturity at harvest, handling, and rain damage. For example, alfalfa at early bud may have 600 g kg^{-1} leaves while at early flower, the leaf share declines to 450 g kg^{-1} *(Sheaffer et al., 2000)*. To value alfalfa hay as a biofuel, or as a source of leaf protein, accurate prediction of the proportion of leaf and stem in the hay is necessary *(Halgerson et al., 2004)*. Decreasing protein content is a dilution effect related with the decreasing leaf to stem ratio; the leaves have stable protein content and their protein level is much higher than the protein content in stems. The decline of digestibility is the consequence of two processes: (a) the reduction of the highly digestible component (leaves) because of an increase of the less digestible component (stems) and (b) the decreasing average digestibility of the stem component, with more cell walls (NDF) and lignin *(Veronesi et al., 2010)*.

When determining the nutritive value of alfalfa, ligneous cellulose content should be taken in account in addition to crude protein content. Neutral detergent fiber (NDF) content indicates the intake rate of alfalfa dry matter. The higher the NDF, the lower the alfalfa quality - the content of nutrients is reduced and livestock consumes such alfalfa less readily. In consequence, the livestock grows at a slower rate and the production of livestock products is proportionally reduced. ADF content indicates the potential production energy. Increase in ADF indicates a reduced energy, i.e., reduced quality *(Katić et al., 2008)*. Forms of energy derived from cellulose hay are not effectively used as livestock feed. Because alfalfa plants accumulate increasing rates of stem material in total herbage during growth, the poor quality of stems becomes a greater issue in more mature alfalfa *(Sheaffer et al., 2000)*.

Significant differences were registered in the contents of crude fiber, ADF and NDF that were caused by genetic factors *(Katić et al., 2008)*. Furthermore, *Sheaffer et al. (1998)* obtained significant differences in the contents of NDF and ADF between low-, medium- and high-quality alfalfa cultivars.

The content of minerals in alfalfa fully meets the livestock requirements while the content of fats is low (averaging 3.8 g/kg), and it varies slightly among cultivars *(Katić et al., 2009)*.

The objective of this study was to determine the chemical composition of dry matter in leaves, stems and whole plants of five divergent alfalfa cultivars.

Materials and Methods

Forage quality was studied in five alfalfa cultivars of different geographic origin, developed by different breeding methods and for different purposes: NS Banat ZMS II (Serbia), Ghareh Yon Geh (Iran), Zuzana (Czech Republic), Pecy (France) and RSI 20 (Spain).

Field trials in five replicates were conducted in 2006 at the experiment field of Institute of Field and Vegetable Crops in Novi Sad.

Chemical analyses were performed on samples taken in the third year of crop life (2008). Samples from the first cut were used for a whole plant analysis and from first and second cuts for leaf and stem analyses. Samples for the whole plant analysis were taken from spaced plants of all genotypes. Sample weight was 500 g of green forage. Plant materials were dried at 60˚C for about 48 hours. Chemical analyses were performed in Laboratory for Soil and Agroecology of Institute of Field and Vegetable Crops in Novi Sad. The analyses were carried out using standard methods, namely, the AOCS-approved Ba 6a-05 procedure for crude fiber (CF) and the Filter Bag Technique (Ankom Technology Corp., Fairport, NY). for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). The analyses were performed on an Ankom 2000 Fiber Analyzer (Ankom Technology Corp., NY, USA).

The crude protein content, mineral substances and oil content (g kg^{-1}) were determined using the standard chemical analysis proposed by Kjeldahl. Oil and ash contents were determined only in leaves and stems.

The two-factorial analysis of variance was used, with cultivar as factor A and cut as factor B. The LSD test was used for testing the significance of differences.

Results and Discussion

The studied cultivars differed significantly in crude protein content. Cultivars Pecy, Zuzana and RSI 20 had higher crude protein contents than cultivars NS Banat ZMS II and Ghareh Yon Geh (Table 1).

Table 1. Quality parameters for the first cut in 2008, in whole alfalfa plants

Cultivar	Protein g kg^{-1}		ADF g kg^{-1}	NDF g kg^{-1}	ADL g kg^{-1}	Hemicellulose g kg^{-1} (NDF-ADF)	Cellulose g kg^{-1} (ADF-ADL)
NS Banat ZMS II	202.0		398.0	455.7	107.0	57.7	291.0
Ghareh Yon Geh	184.0		416.7	442.7	113.0	26.0	303.7
Zuzana	206.0		413.0	434.0	92.3	21.0	320.7
Pecy	209.0		397.7	421.3	99.0	23.7	298.7
RSI 20	206.0		388.0	412.0	92.0	24.0	296.0
Average	201.0		402.7	433.1	100.7	30.5	302.0
CV %	6.0		2.5	2.3	3.1	9.5	3.8
0.05	23.0		19.2	18.7	5.9	5.4	21.7
LSD 0.01	33.0		28.0	27.2	8.6	7.9	31.6

The cultivars differed significantly in the fiber content for the whole plant. Cultivars NS Banat ZMS II and Ghareh Yon Geh had highest contents of neutral fibers, i.e., highest dry matter intake rates. Cultivar Ghareh YonGeh had highest contents of ADF and ADL. At the whole plant level, cultivar RSI 20 had lowest

NDF, ADF and ADL contents and the highest energy value of dry matter (Table 1). Also at the whole plant level, the coefficients of variation for crude protein and hemicellulose contents were 6% and 9.5%, respectively, while the other quality components showed relatively low variations (2.5% - 3.8%).

The analysis of quality components in the leaf emphasized the importance of foliage preservation during storage of alfalfa hay or haylage. Leaves accumulate high contents of crude proteins and minerals (287.7 and 110.7 g kg^{-1}, respectively). Also, the fiber content (cellulose, hemicellulose, NDF, ADF and ADL) is significantly lower in alfalfa leaves than in stems (Table 2). In this study, cultivar Pecy had the highest protein content in leaves (297.4 g kg^{-1}), which was significantly higher than those found in cultivars Ghareh Yon Geh and NS Banat ZMS II (272.3 and 279.3 g kg^{-1}, respectively). Highest contents of NDF, ADF and minerals in leaves were found in cultivar Ghareh Yon Geh (Table 2). There were no significant differences among the cultivars in the ligneous cellulose complex (except for the NDF content) and oil contents in leaves. The cultivars differed significantly in the content of minerals in leaves (Table 2). The lowest content of minerals was recorded in cultivar Pecy (99.1 g kg^{-1}).

Table 2. Quality parameters for the first and second cut in 2008 (average), in alfalfa leaves

Cultivar	Protein g kg^{-1}	NDF g kg^{-1}	ADF g kg^{-1}	ADL g kg^{-1}	Oil g kg^{-1}	Ash g kg^{-1}	Hemicellulose g kg^{-1} (NDF-ADF)	Cellulose g kg^{-1} (ADF-ADL)
NS Banat ZMS II	279.3	173.7	137.6	45.4	20.8	108.8	36.0	92.2
Ghareh Yon Geh	272.3	190.4	153.8	38.5	17.8	125.1	36.5	115.3
Zuzana	294.7	176.6	153.1	33.3	23.9	102.0	23.5	119.8
Pecy	297.4	178.8	142.8	37.6	24.2	99.1	36.0	105.2
RSI 20	291.9	171.3	145.2	40.5	21.8	118.4	26.1	104.6
Average	287.1	178.1	146.5	39.1	21.7	110.7	31.6	107.4
CV %	7.1	1.0	2.7	20.2	16.8	4.6	11.5	6.4
0.05	18.4	14.9	29.4	21.9	10.0	13.9	25.7	38.3
LSD 0.01	24.7	20.0	39.5	36.3	13.4	18.7	34.5	51.4

The coefficients of variation for the contents of crude protein, cellulose, hemicellulose and oil in leaves of the analyzed alfalfa genotypes were 7.1%, 6.4% 11.5% and 16.8% respectively. Highest variations in leaves were registered for the content of ADL (20.2%). The coefficients of variation for NDF, ADF and ash in leaves were low.

Quality traits of the stem indicated that alfalfa stems had a significantly lower nutritional value than leaves (Table 3), which is consistent with a study of *Sheaffer et al. (2000)*. There were no significant differences in the contents of NDF and ADF, hemicellulose (NDF-ADF), cellulose (ADF-ADL), oil and minerals in stems of the analyzed cultivars.

High coefficients of variation were obtained for the contents of oil and hemicellulose in the stem (27.2% and 19.8%). The contents of ADF varied

between 5% and 10%. Low variations were obtained for the contents of NDF and ADL in the stem (Table 3).

Table 3. Quality parameters for the first and second cut in 2008 (average), in alfalfa stems

Cultivar	Protein g kg^{-1}	NDF g kg^{-1}	ADF g kg^{-1}	ADL g kg^{-1}	Oil g kg^{-1}	Ash g kg^{-1}	Hemicellulose g kg^{-1} (NDF-ADF)	Cellulose g kg^{-1} (ADF-ADL)
NS Banat ZMS II	109.3	583.3	504.9	118.4	8.1	48.7	78.3	386.6
Ghareh Yon Geh	103.8	587.2	500.9	115.9	11.1	49.6	86.4	385.0
Zuzana	114.3	588.2	500.8	109.0	9.9	46.9	87.5	391.8
Pecy	105.4	622.6	529.3	113.9	7.8	44.3	93.3	415.4
RSI 20	109.0	592.0	513.3	114.1	10.2	48.5	78.7	399.2
Average	108.4	594.7	509.8	114.3	9.4	47.6	84.8	395.6
CV %	4.2	2.1	4.8	2.3	27.2	9.0	19.8	6.0
0.05	10.3	35.8	46.1	8.2	8.7	5.9	52.1	43.2
LSD 0.01	13.9	48.0	61.8	11.0	11.7	7.9	70.0	58.5

Nonsignificant differences existed among the cultivars in crude protein and ADL contents. The highest protein content in the stem was registered in cultivar Zuzana (114.3 g kg^{-1}), the lowest in cultivar Ghareh Yon Geh (103.8 g kg^{-1}). The highest ADL content was found in cultivar NS Banat ZMS II (118.4 g kg^{-1}), the lowest in cultivar Zuzana (109.0 g kg^{-1}). The difference between them was significant. High lignin content in alfalfa plants increases their resistance to lodging; however, lignin is a major factor that limits cell wall digestibility because it inhibits the digestibility of polysaccharides *(Katic et al., 2008)*.

The coefficients of variation indicated that the variability of the quality traits was not the same in the plant, stems and leaves. Based on variability indicators, the contents of crude protein and cellulose should be monitored in the whole plant. The contents of crude protein, ADL, oil, ash and hemicellulose should be monitored in leaves. However, contents of oil, ash, hemicellulose and cellulose should be monitored in stems.

The results for the quality traits of alfalfa leaves, stems and whole plants obtained in this study are in agreement with those from previous studies *(Sheaffer et al. 2000)*.

The results obtained in this paper confirm those of *Veronesi et al. (2010)*, who claimed that there exist subtle differences between genotypes in the main quality parameters and that alfalfa breeding for increased nutritional value is a viable option.

Conclusion

The studied alfalfa cultivars varied in the contents of proteins, cellulose, hemicellulose, NDF, ADF, ADL, and minerals depending on genotype and plant part.

Significant differences existed among the alfalfa cultivars in the nutritional components at the level of the whole plant.

High contents of crude proteins, oil and minerals were registered in alfalfa leaves, while significantly high fiber content (NDF, ADF, ADL, cellulose, hemicellulose) were registered in alfalfa stems.

The obtained results indicated that the quality parameters varied at the levels of the whole plant, leaves and stems.

The results confirmed the importance of alfalfa breeding for high nutritional value. They also indicated that the studied cultivars were a significant source of desirable genes needed for development of alfalfa cultivars with improved quality.

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Hemijski sastav lišća i stabljika divergentnih sorti lucerke

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Rezime

Lucerka je višegodišnja vrsta koja daje veći prinos proteina po jedinici površine od bilo koje ratarske kulture, pa je zbog toga veoma poželjna za proizvodnju sena i u ishrani domaćih životnja (posebno preživara). Cilj rada je bio da se odredi hemijski sastav suve materije lucerke kod pet divergentenih sorti lucerke u celoj biljci kao i u listu i stabljikama. Lucerka varira u sadržaju proteina, celuloze, hemiceluloze, NDF, ADF, ADL, i mineralnih materija u zavisnosti od genotipa i biljnog dela. Postoje značajne razlike u komponentama hranljive vrednosti lucerke između sorti što ukazuje da oplemenjivanje na veći kvalitet lucerke ima smisla.

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BIOLOGICAL AND GENETICAL CHARACTERISTICS OF HYBRID MAIZE KNEZA 683A

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Original scientific paper

Abstract: The basic biological and economic properties of the hybrid maize Kneza 683A, created in Maize Research Institute – Kneza, R. Bulgaria by the method of interline hybridization are briefly described. The hybrid is acknowledged during year 1994 after two years variety-testing in SVTC (State Variety-Testing Commission) with standards American hybrids of maize H 708 and Ivana. During the period of testing, in year 1992 and 1993, in condition without irrigation, the average enlargement of yield on hybrid maize Kneza 683A (8 140 kg/ha), in comparison with hybrid maize H 708 (7 210 kg/ha) was 11, 42% and with hybrid maize Ivana (7610 kg/ha) was 6,51%. In conditions with irrigation, the average enlargement of yield on hybrid maize Kneza 683A (10 610 kg/ha), compared to first standard H 708 (9 890 kg/ha) was 6,78%, and compared to second standard Ivana (10 570 kg/ha) was 0,37%.The hybrid maize Kneza 683A, was examined in year 1994 and 1995 in R. Macedonia in conditions with irrigation and in R.Bulgaria, without irrigation. In comparison with standard american hybrid of maize H 708 (11 233 kg/ha), in our country the average enlargement of yield on the hybrid maize Kneza 683A (11 556 kg/ha) was 2,79%, and in R. Bulgaria was 8,04%. In the examination in year 2006 in R. Macedonia in conditions with irrigation, the average enlargement of yield on the hybrid maize Kneza 683A (11 600 kg/ha) in comparison with standard Kneza 630 (8 830 kg/ha) was 8,04% and hybrid Kneza 621 (11 200 kg/ha) was 3,44%. After examination from SVTC, in year 2007, the University "Goce Delcev" Stip, Institute for Southern Crops in Strumica in our country have registered the hybrid on the sort list. In 2008 was organised seed production on area of 1 ha and there was yield of 2000 kg/ha, which means that although the late sowing there was good yield of seed. In field conditions the hybrid is practically resistant to the economically important disease by the maize. The seed production of the hybrid maize Kneza 683A carry out with simultaneously sowing of the parental components using cytoplasmic male sterility (CMS) and fully restoration of fertility in hybrid generation. The hybrid maize Kneza 683A successfully shift the late Bulgarian and foreign hybrids (group 600 by FAO), since is more drought-resistant and considerably exceeds them by grain yield and silage mass from a unit of area.

Key words: maize, hybrid, biological properties, grain yield, fresh mass

Introduction

The hybrid maize Kneza 683A is from the group of late hybrid maize, group 600 according to classification of UN (FAO). Created in the Maize Research Institute-Kneza, R. Bulgaria, the authorial collective, with hybridization of the original mutant line K 4640 B and American line Mo 17. The aim of our examinations was introduction of Bulgarian maize hybrids in R. Macedonia, which will give higher yields of grain and green mass - silage of the established standards (american, bulgarian and serbian hybrids).

In order to introduce the scientific community and agribusiness with productive properties of the hybrids and the possibilities for exploitation in various conditions of cultivation were organaised the trials and shown the biological and genetical characteristics of Hybrid maize Kneza 683A.

Materials and Methods

Hybrid obtained by crossing the mutagenic line ♀ K 4640B with the American line ♂ Mo 17. K4640B line is created by chemical mutagenic action of the Maize Research Institute -Kneza, R. Bulgaria. The material is obtained from the Maize Research Institute-Kneza, R. Bulgaria Prior culture of the maize was wheat.

Monitored are the basic biological and economic characteristics of the hybrid maize Kneza 683A. Assessment is made of the properties of productivity, based on measurements made after the harvest of each repetition of the test hybrid. It is made evaluation of resistance of onslaught of diseases and insects (H. Turcikum, U. maidis, Fusarium spp, O. nubilalis). Evaluation of lying of the plants and broken plants was performed before harvesting. After harvesting is done determining of the moisture in grain and yield in kg/ha with 14 % moisture.

The examination of the hybrid was conducted in the competition sort trials (CST) in the period from year 1989 to 1997, in the Maize Research Institute-Kneza in 1993 - 1994 and in 1993 – 1994 in SVTC (State Variety-Testing Commission) in R. Bulgaria. Experiments are placed after the block method and the method of latin rectangle in 3 and 4 repetitions with the size of the experience parcel 10 m^2. Schemes for field trials are used according to *Shanin (1977)* and *Barov (1982).* Dispersion analysis of the results of the trials was done after *Shanin (1977)*.

The examinations of the hybrid maize 683A in R.Macedonia are made in year 1994 and 1995 on area of ZK "Pelagonia" – R. Macedonia in conditions with irrigation and on area at the Maize Research Institute-Kneza, R. Bulgaria without irrigation. Then in year 2006, they are made on area at the Institute of Southern Crops – Strumica, R. Macedonia in conditions with irrigation. The experiments are performed after the method of random block system, in 5 repetitions with the size of the experience parcel of 10 m^2.After examination from SVTC (State Variety-Testing Commission) in R. Bulgaria, in year 2007, the University "Goce Delcev" Stip, Institute for Southern Crops in Strumica in our country have registered the hybrid on the sort list. In 2008 was organised seed production on area of 1 ha.

Results and Discussion

The hybrid maize Kneza 683A is a simple cross-line hybrid, created in the Maize Research Institute-Kneza, R. Bulgaria, in year 1989 by hybridization on the mutant line K4640B and the american line Mo 17. Hybrid maize Kneza 683A is a late hybrid, according to the FAO classification belongs to the group of maturity (600-699), which is evident from the results obtained during the test. The period from sprouting to silking is 72 days without irrigation in R. Bulgaria and 74 days with irrigation in R. Macedonia.

Period from sprouting to physiological maturity is 133 days without irrigation in R. Bulgaria and 138 days with irrigation in R. Macedonia.

The hybrid has a high, healthy stem, without anthocyanins coloration at the beginning of the leaves. Silk is light - pink. Cob is like a cone, 16-18 rows of grain and with a medium length from 24-27 cm. Spindle is with anthocyanins color, the shape of the grain is like a tooth, the color of the grain of the forehead is yellow and with very dark color in the basis. The mass of 1000 grains is 360-380 grams, and randeman durning the crumbling of the cobs is 85-86%.

Table 1 – Biological characheristics of hybrid maize Kneza 683A

Simple hybrid	M.L. K 4640B x USA Mo 17	
FAO	600 – 699	
	With irrigation	Without irrigation
Sprouting – sliking	72 days	74 days
Sprouting - physiological maturity	133 days	138 days
Stalk	High	
Colour of the silk	light – pink	
Form of the cob	like a cone	
Length of the cob in sm	24-27	
Type of grain	like a tooth	
Mass on 1000 grains in g	360 – 380	
Randeman on the grain in cob (%)	85-86	

The hybrid is acknowledged during year 1994 after two years variety-testing in SVTC (State Variety-Testing Commission) with standards American hybrids of maize H 708 and Ivana.

During the period of testing from SVTC (State Variety-Testing Commission) in R. Bulgaria 1992 and 1993 in conditions without irrigation, the average enlargement of yield on hybrid maize Kneza 683A (8 140 kg/ha), in comparison with american hybrid maize H 708 is 11,42% or 7 210 kg/ha and with Ivana is (6,51% or 7 610 kg/ha). In conditions with irrigation, the average enlargement of yield on hybrid maize Kneza 683A (10 610 kg/ha), compared to first standard H 708 is 6,78% or 9 890 kg/ha and compared to the second standard Ivana is 0,37% or 10 570 kg/ha *(Genov. and Genova, 2005)*.

Table 2. Average results for yield of grain on hybrid maize K-683A, in comparison with standards H 708 St (1) and Ivana St (2) in production year 1992-1993, in the period of examination in SVTC in R. Bulgaria (without irrigation).

Hybrids	FAO (group)	Average Yield of grain kg/ha	Comparison with St in %
H-708 St (1)	700	7210	100
Ivana St (2)	700	7610	100
K-683A	600-699	8140	112, 9 (1), 106, 96 (2)

In Table 2 are given the average results for yield of grain on hybrid maize K-683A, in comparison with standards H 708 St (1) and Ivana St (2) in production year 1992-1993, in the period of examination in SVTC in R. Bulgaria (without irrigation).

Table 3. Average results for yield of grain on hybrid maize K-683A, in comparison with standards H 708 St (1) and Ivana St (2) in production year 1992-1993, in the period of examination in SVTC in R. Bulgaria (with irrigation).

Hybrids	FAO (group)	Average Yield of grain kg/ha	Comparison with St in %
H-708 St (1)	700	9890	100
Ivana St (2)	700	10570	100
K-683A	600-699	10610	107, 28 (1), 100, 38 (2)

In Table 3 are given the average results for yield of grain on hybrid maize K-683A, in comparison with standards H 708 St (1) and Ivana St (2) in production year 1992-1993, in the period of examination in SVTC in R. Bulgaria (with irrigation).

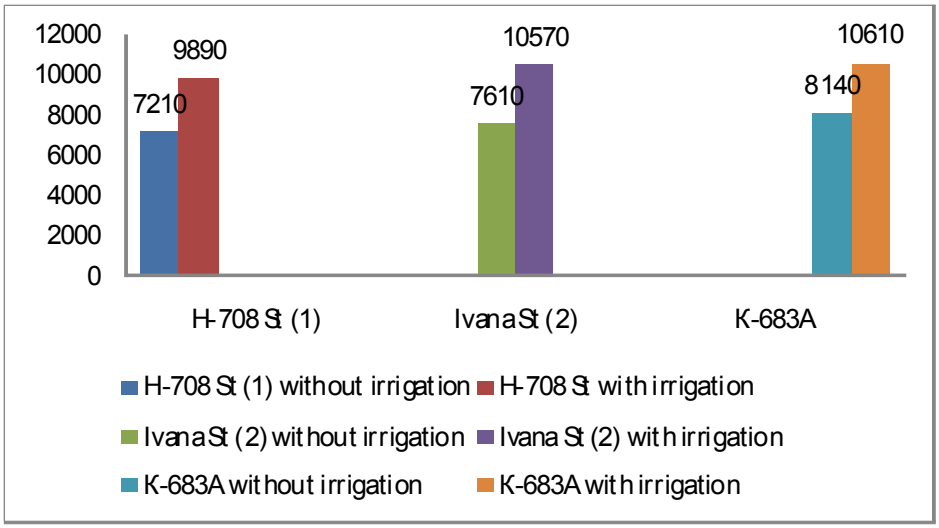

Figure 1. The results from table 2 and 3 are shown, for yield of grain on hybrid maize K-683A, in comparison with standards H 708 St (1) and Ivana St (2) in production year 1992-1993, in the period of examination in SVTC in R.Bulgaria (without and with irrigation).

The hybrid maize 683A, was examined in year 1994 and 1995, on area of ZK "Pelagonia" R. Macedonia in conditions with irrigation and on area at the Maize Research Institute-Kneza, R. Bulgaria, without irrigation. In comparison with standard american hybrid maize H 708 (11 233 kg/ha), in our country the average enlargement of yield on hybrid maize Kneza 683A is for 2,79% or 11 556 kg/ha, while in R. Bulgaria in comparison with the standard H-708 (8120 kg/ha), average enlargement on the yield of hybrid maize Kneza 683A is for 8,04% or 8830 kg/ha *(Gacovski, 1998)*.

Table 4. Average results for yield of grain on hybrid maize K-683A, in comparison with the standard H 708 St (1) in production year 1994-1995, in the period of examination in ZK "Pelagonia" R. Macedonia (with irrigation).

Hybrids	FAO (group)	Average Yield of grain kg/ha	Comparison with St in %
H-708 St (1)	700	11233	100
K-683A	600-699	11556	102, 87(1)

In Table 4 are given the average results for yield of grain on hybrid maize K-683A, in comparison with the standard H 708 St (1) in production year 1994-1995, in the period of examination in ZK "Pelagonia" R. Macedonia (with irrigation).

Table 5. Average results for yield of grain on hybrid maize K-683A, in comparison with the standard H 708 St (1) in production year 1994-1995, in the period of examination in ZK "Pelagonia" R. Macedonia (without irrigation).

Hybrids	FAO (group)	Average Yield of grain kg/ha	Comparison with St in %
H-708 St (1)	700	8120	100
K-683A	600-699	8830	108, 74 (1)

In Table 5 are given the average results for yield of grain on hybrid maize K-683A, in comparison with the standard H 708 St (1) in production year 1994-1995, in the period of examination in ZK "Pelagonia" R. Macedonia (without irrigation).

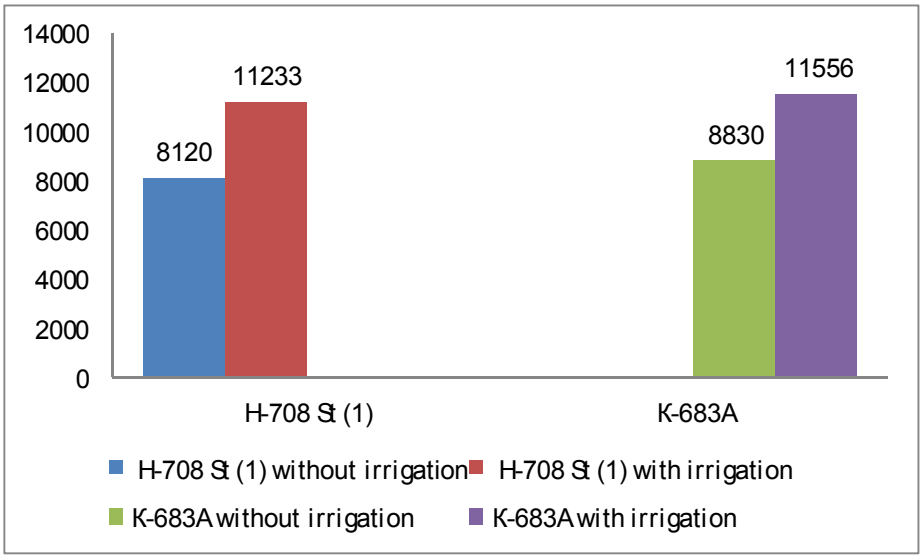

Figure 2. The data from table 4 and 5 are shown, for yield of grain on hybrid maize K-683A, in comparison with standard H 708 St (1), in production year 1994-1995, in the period of examination on area of ZK "Pelagonia" R. Macedonia (without and with irrigation).

In the examination in year 2006 in R. Macedonia, on area at the Institute for southern crops- Strumica, R. Macedonia, in conditions with irrigation, the average enlargement of yield on the hybrid maize Kneza 683A (11 600 kg/ha) in comparison with standard Kneza 630 is for 8,62% or 10 600 kg/ha and hybrid Kneza 621 for 3,44% or 11 200 kg/ha *(Gacovski, 2006)*.

Table 6. Average results for yield of grain on hybrid maize K-683A, in comparison with the standard Kneza 630 (1) and Kneza 621 in production year 2006, on area at the Institute for Southern Crops in Strumica, R.Macedonia (with irrigation).

Hybrids	FAO (group)	Average Yield of grain kg/ha	Comparison with St in %
Kneza 630 (1)	650	8830	100
Kneza - 683A	600-699	11600	131, 37
Kneza 621	650	11200	126, 84

In Table 6 are given the average results for yield of grain on hybrid maize K-683A, in comparison with the standard Kneza 630 (1) and Kneza 621 in production year 2006, on area at the Institute for Southern Crops in Strumica, R. Macedonia (with irrigation).

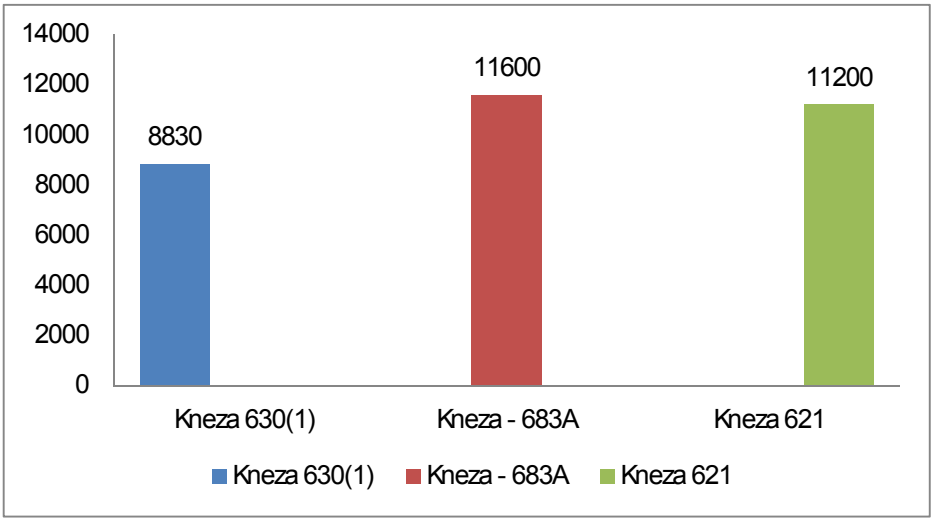

Figure 3. The data from table 6 are shown, for yield of grain on hybrid maize K-683A, in comparison with standard Kneza 630 (1) and Kneza 621, in production year 2006, on area at the Institute for Southern Crops in Strumica, R.Macedonia) (with irrigation).

After examination from SVTC (State Variety-Testing Commission) in R. Bulgaria in year 2007, the University "Goce Delcev" Stip, Institute for Southern Crops in Strumica in our country have introduced and registered the hybrid on the sort list. In 2008 was organised seed production on area of 1 ha and there was yield of 2000 kg/ha, which means that although the late sowing there was good yield of seed.

The optimal sowing period is from 15 to 30 April. Fertilizattion N - 160 kg/ha, P - 90 kg/ha и K - 90 kg/ha, then on time proper protection from weeds and

pests is needed and 400 mm rainfalls with uniform disposition durning year for obtaining normal progress and maturity.

Optimal solidity on the hybrid maize Kneza 683A durning production in condition with irrigation is 60 000 plants/ha, while without irrigation is 40 000 plants/ha.

From the hybrid maize Kneza 683A, in conditions with irrigation is obtained 15 000 – 20 000 kg/ha grain, while without irrigation with normal disposition of rainfalls durning vegetation is obtained 13 000 – 15 000 kg/ha.

Because of the strong vegetative develop this hybrid is suitable for production of forage. Harvest durning the use of furage is suitable to be made in waxed maturity.

In field conditions the hybrid is practically resistant to the economically important disease by the maize: leaves carp or withering, simple glamnica, decomposing of stalk, furasioza of the cobs and simple mosaic.

Tehnology of seed production of hybrid maize Kneza 683A is carry out with simultaneously sowing of the parental components. Hybrid is using cytoplasmic male sterility (CMS) with fully restoration of fertility and after desire of the producers, seed production may be carry out with or without removing on taseles of motherly rows.

The big breed coefficient of the mother give a possibility for obtaining yield from 4 000 – 6 000 kg/ha standard hybrid seed.

Scheme of sowing on the hybrid lot is 6:2 or 4:2. Restoration of seed production on the hybrid is made in the Maize Research Institute-Kneza.

The hybrid maize Kneza 683A successfully shift the late maize hybrids, especially the American hybrids, since is more drought-resistant and considerably exceeds them by grain yield and silage mass from a unit of area.

Conclusion

1. Hybrid maize Kneza 683 A, after the property yield of grain in (kg/ha), considerably exceeds the hybrids from the late maternity group (FAO 600), used as standards durning the period of examination, after which define the hybrid suitable for seed production.

2. Examination of yield from the whole plant shows that hybrid maize Kneza 683 A is among the best hybrids for silage production.

3. Hybrid maize Kneza 683A, is resistant to the most important diseases by the maize in our country.

4. Seed production is suitable because of the high breed coefficient, simultaneously sowing of the parental components and removing of the tasele at female -♀ mother component.

5. Seed production is profitable because of the high breed coefficient, simultaneously sowing of the parental components and removal of the taseling at mother component.

Biološke i genetske karakteristike hibrida kukuruza Kneža 683a

Ž. Gacovski, G. Cilev

Rezime

U radu su opisane osnovne biološke i ekonomske karakteristike hibrida kukuruza Kneža 683a stvorenog u Institutu za kukuruz – Kneža u R. Bugarskoj.

Hibrid je priznat u toku 1994. godine, nakon dve godine testiranja, od strane državne službe za testiranje sorata, sa standardima, američkim hibridom H 708 i Ivanom.

U toku perioda testiranja u 1992. i 1993. godini u uslovima bez navodnjavanja hibrid Kneža 683A (8 140 kg/ha), u odnosu na hibrid H 708 (7 210 kg/ha) postigao je veći prinos za 11,42%, a u odnosu na hibrid Ivana (7610 kg/ha) za 6,51%. U uslovima navodnjavanja prosečni prinosi hibrida Kneža 683A (10 610 kg/ha), u poređenju sa prvim standardom H 708 (9 890 kg/ha) bili su veći za 6,78%, a u poređenju sa drugim Ivana (10 570 kg/ha) za 0,37%.

Hibrid kukuruza Kneža 683A, istraživan je u 1994. i 1995. godini u R. Makedoniji u uslovima navodnjavanja i u R. Bugarskoj u uslovima bez navodnjavanja. U poredjenju sa standardom, američkim hibridom H 708 (11 233 kg/ha), u našoj zemlji, Kneža 683A (11 556 kg/ha) je ostvario prinos veći za 2,79%, a u R. Bugarskoj za 8,04%. U ispitivanjima u 2006. godini u R. Makedoniji u uslovima navodnjavanja prosečno povećanje prinosa Kneža 683A (11 600 kg/ha) bilo je za 8,04% veće od standarda, hibrida Kneza 630 (8 830 kg/ha) i za 3,44% od Kneža 621 (11 200 kg/ha).

Nakon istraživanja od strane SVTC u 2007. godini, Univerzitet "Goce Delcev", Institut za južne useve u Strumici je registrovalo hibrid na sortnoj listi. U 2008. godini organizovana je semenska produkcija i dobijen je prinos od 2000 kg/ha, što znači da i pored kasne setve postoji dobar prinos semena.

U poljskim uslovima hibrid je otporan na sve ekonomski važne bolesti kukuruza. Proizvodnja semena hibrida Kneža 683A zasniva se na simultanoj setvi roditelja korišćenjem citoplazmatične muške sterilnosti – CMS, i potpunom obnavljanju plodnosti generacije hibrida.

Hibrid Knežza 683A uspešno je zamenio kasno bugarske i druge strane hibride (iz grupe FAO 600), pošto je više otporan na sušu, daje veći prinos zrna i više silažne mase sa jedinice površine.

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STAND DENSITY EFFECTS ON BIRDSFOOT TREFOIL HERBAGE YIELD GROWN FOR COMBINED USAGE

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Original scientific paper

Abstract: Birdsfoot trefoil grown for seed (cv. Bokor) was examined in agroecological conditions of Western Serbia during period 2007-2009. The goal was to determine herbage yield which could be achieved growing seed crop, using different interrow spacing (12.5, 25 and 50 cm) and various seeding rates (2, 4 and 8 kg ha^{-1}). The highest hay yield was obtained in the third production year at the narrowest spacing and using the highest seeding rate (5.26 t ha^{-1}). Total hay production during 3 years using birdsfoot trefoil seed crop for herbage yield ranged from 9.11 to 14.83 t ha^{-1} depending on row spacing and from 10.74 to 13.2 t ha^{-1} depending on seeding rate. The highest stand density (8 kg ha^{-1} seeding rate and 12.5 cm interrow spacing) produced the highest herbage yield of birdsfoot trefoil in the combined usage (for forage and seed production).

Key words: birdsfoot trefoil, row spacing, seeding rate, herbage yield

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.) belongs to perennial legumes which is the special group of plants important for forage production. It takes the most important place in grass-legume mixtures for establishing artificial grasslands in the hilly-mountainous regions of Serbia. As pure crop, it is grown locally only on small areas.

It may grow in very different agroecological conditions, on almost all types of soil, with very wide range of pH value, from 4 to 9 *(Marvin, 2004)*. On soils of lower quality, birdsfoot trefoil has ignificant role in providing protein component in forage. However, in favourable conditions of soil and climate, it considerably falls behind the yield of alfalfa *(Gotlin and Čižek, 1955)*. During vegetation period it could be cut for herbage biomass three times, and under favourable conditions even four times. Average yield of biomass (green fodder) ranges from 35-40 t ha^{-1}, and of hay 8-10 t ha^{-1} *(Vučković, 2004)*. Some studies suggest that it is possible to

achieve the yields of pure birdsfoot trefoil hay of 6-14 t ha^{-1}, and in the mixture with grasses 10-17 t ha^{-1} *(Bullard and Crawford, 1995)*.

Birdsfoot trefoil has good palatability, it contains high percentage of protein and it is easily digestible for cattle, sheep and horses *(Sareen, 2004)*. The hay of birdsfoot trefoil is of excellent quality, with high content of crude protein *(Halling et al., 2002)* and plenty of B-carotene and vitamin C. Quality of forage does not change with development stages so fast as it is the case with other legumes. It contains 19.68-25.75% crude protein and only 18.38-24.20% of crude cellulose in the flowering stage *(Ocokoljić et al., 1978)*. While studying morphological and nutritional characteristics of wild populations of birdsfoot trefoil, *Vučković et al. (2007)* concluded that Serbian populations are more superior according to their chemical characteristics and nutritional values as compared to populations from other parts of the world. Presence of condensed tannins in the leaves and stem prevents bloating of ruminants, so that birdsfoot trefoil does not cause bloating of cattle while grazing *(Beuselinck and Grant, 1995)*. By comparing birdsfoot trefoil and alfalfa, grazed by sheep and lambs, *Douglas et al. (1995)* established greater production of wool in sheep and weight in lambs, when using birdsfoot trefoil in their nutrition. Recent studies have shown that sheep that have used the hay of birdsfoot trefoil in their diet have less problems with parasitic infections *(Marley et al, 2002)*. Strongly developed root system of birdsfoot trefoil binds well and protects soil against erosion. Considering the fact that the process of biological fixation of nitrogen takes place indirectly, through symbiotic bacteria, birdsfoot trefoil takes significant place in sustainable agricultural and organic production *(Tomić, 2007)*.

Forage species have utmost importance in the development of cattle breeding in hilly-mountainous region. One of conditions for faster expansion of this species is providing of sufficient quantity of high-quality seeds. There are favourable conditions in Western Serbia for seed production of this forage legume *(Petrović, 2011)*. Crops intended for seed production make certain quantity of forage. Due to variation of seed yields, and its price at the market, there is an issue of possible increase of birdsfoot trefoil profitability, by combined use, where certain yield of forage is made, in addition to seed production. The aim of our studies was to establish the quantity of forage that can be obtained by growing the birdsfoot trefoil crops for combined use, when establishing on various interrow spacings and with various seeding rates.

Materials and Methods

The field experiment was carried out in Western Serbia, near the city of Šabac, in the period 2007-2009. Seeding of birdsfoot trefoil (cv."Bokor") was carried out in spring 2007 in the completely randomized block design in four replications, with the basic plot of 10 m^2 (2x5m). The way of seeding (factor A) with three interrow spaces (12.5, 25 and 50 cm) and the amount of seeds for

seeding (factor B) with three seeding rates (8, 4 and 2 kg ha^{-1}) were tested. In the first experimental year, the seed was obtained from the first cutting, and then the production of forage was measured from the second and the third, while in the second and the third year of birdsfoot trefoil life, the second cutting was used for seeds, and the first and the third one was used for forage. The yield of green biomass was established in the stage of bud emergence early stage of flowering, from each plot. After drying of biomass and establishment of drying factor, the yield of dry material per hectare was established. Obtained data were processed in variation-statistical way, by variance analysis, and evaluation of significant differences were established by LSD test.

Agro-ecological conditions. The soil on which the experiment was carried out belongs to pseudogley. According to results of chemical analysis (Table 1) the soil is carbonate-free, in the humus ploughland horizon of acid reaction, with decreasing acidity trend in parallel with increasing depth. It belongs to low and low slightly humus soil, poor in total nitrogen. The soil is poor in terms of contents of easily available phosphorus, and it is medium rich in easily available potassium.

Table 1. Chemical properties of soil

Depth (cm)	pH		Humus (%)	Total N (%)	Content in mg/100g of soil	
	H_2O	nKCl			P_2O_5	K_2O
0-30	5,45	4,50	1,84	0,092	8,2	15,0
30-50	5,80	4,73	1,61	0,088	6,1	13,1
50-70	6,15	4,97	0,85	0,064	5,0	13,7

Table 2. Precipitation and mean monthly temperatures for experimental period

Month	Temperatures (^{0}C)				Precipitation (mm)			
	2007	2008	2009	1977-2006	2007	2008	2009	1977-2006
I	5,2	1,9	-1,5	- 0,2	40,2	27,6	42,1	38,2
II	5,8	4,3	2,4	1,6	49,5	5,4	30,5	34,3
III	8,6	7,6	7,4	6,5	40,3	57,4	38,2	39,2
IV	13,0	12,9	14,3	11,3	0,0	52,4	12,0	49,5
V	18,5	18,3	18,4	16,8	49,0	42,4	44,1	55,3
VI	22,0	21,5	19,5	19,7	65,2	58,1	81,3	85,5
VII	22,6	21,7	22,3	21,2	38,7	61,0	24,0	62,5
VIII	22,3	21,4	22,6	20,8	42,5	22,7	42,3	56,9
IX	14,3	15,3	15,0	16,4	73,4	76,4	70,1	49,3
X	10,4	12,9	11,2	11,6	68,0	12,6	77,3	50,8
XI	3,7	7,9	5,1	5,5	62,6	48,8	80,0	52,6
XII	0,3	3,7	0,5	1,1	32,2	66,2	47,0	44,4
Total					561,6	531,0	588,9	618,5
Average	12,2	12,5	11,4	11,0				

Based on data of weather station in Sremska Mitrovica (Table 2), it is seen that average air temperatures in the experimental years were higher than the several years' average. All experimental years had even sums of precipitation, but somewhat lower as compared to the several years' average. Due to higher temperatures and lower precipitation, short periods of drought in 2007 and 2009 occurred in April and July, and in 2008 in August and October.

Results and Discussion

In all experimental years (Table 3), the way of seeding and seeding rate had high influence on the yield of hay per cuts. With increase of width of interrow space, the average yield of hay decreased, so that the highest yields were achieved at the minimum spacing of 12.5 cm. With decrease of seeding rate, the average yield of forage decreased so that the highest yields were established on the variant with the highest seeding rate of 8 kg ha^{-1}, i.e. the highest density of crops.

Table 3 Effect of interrow spacing and seeding rate on birdsfoot trefoil herbage yield (DM) in two herbage cuts during seed production (2007-2009)

	Herbage yield (t ha^{-1})					
	2007		2008		2009	
Interrow spacing A	II cut	III cut	I cut	III cut	I cut	III cut
12.5 cm	1.75[a]*	0.87[a]	4.47[a]	1.18[a]	5.02[a]	1.54[a]
25 cm	1.61[b]	0.67[b]	3.53[b]	0.96[b]	4.11[b]	1.10[b]
50 cm	1.51[c]	0.50[c]	2.48[c]	0.59[c]	3.24[c]	0.79[c]
LSD 0.05	*0.046*	*0.045*	*0.11*	0.059	*0.17*	*0.042*
Seeding rate B						
8 kg ha^{-1}	1.80[a]	0.85[a]	3.75[a]	1.07[a]	4.43[a]	1.30[a]
4 kg ha^{-1}	1.69[b]	0.67[b]	3.51[b]	0.91[b]	4.04[b]	1.14[b]
2 kg ha^{-1}	1.38[c]	0.51[c]	3.22[c]	0.75[c]	3.89[b]	0.99[c]
LSD 0.05	*0.046*	*0.045*	*0.11*	0.059	*0.17*	*0.042*
Average	1.62	0.68	3.49	0.91	4.12	1.14

*Means in columns followed by the same letter are not significantly different by Fisher's protected LSD values (P=0.05)

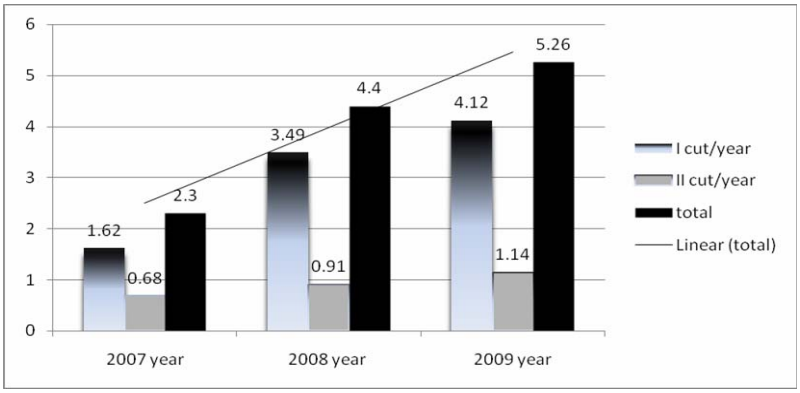

Figure 1. Herbage yield of birdsfoot trefoil during seed production, t ha^{-1} (2007-2009)

By observing forage yields per cuts, earlier cuts were higher than the later ones, which is as expected (Figure 1). Total production of hay per experimental years differed to significant extent. In the year of establishment, total hay production was 2.30 t ha^{-1}, in the second year it was 4.40 t ha^{-1}, and in the third year it was 5.26 t ha^{-1}. Production of forage was directly dependant on the age of crops and it increased with years of crop use. The birdsfoot trefoil crop was in the third year of production in 2009 with considerably developed plants, so that it achieved higher yield of forage per hectare as compared with the first and the second experimental year. This is the reason why in 2009 the highest annual hay yield (6.56 t ha^{-1}) was achieved at the narrowest interrow spacing (12.5 cm) and of 5.73 t ha^{-1} by using the highest seeding rate (8 kg ha^{-1}). Total hay production in the three-year period of using birdsfoot trefoil crop ranged from 9.11 to 14.83 ha^{-1} depending on the interrow spacing and from 10.74 to 13.2 ha^{-1} depending on the seeding rate.

In previous studies of impact that the way of seeding and seeding rate make on the yield of birdsfoot trefoil crop, the authors point out the advantage of narrow row seeding compared with wide row seeding. So, *Miladinović (1964)* obtained the highest yield of hay yield on the shortest interrow space of 30 cm, which in the first cutting was 5.42 t ha^{-1} of hay. *Gatarić (1988)* achieved the highest average hay yield of 5.92 t ha^{-1}, in the first cut by denser seeding, i.e. by broadcast seeding with 20 kg ha^{-1} of seeds. In the studies of *Balan et al. (2002)* the average yields of dry matter of tested genotypes of birdsfoot trefoil ranged from 5.4 to 5.8 t ha^{-1}. Forage yields obtained in the third year of our experiments on the minimum rows and at the highest seeding rate, match the previously mentioned data from literature.

By growing birdsfoot trefoil on eroded soil *Mijatović (1975)* obtained the hay yield of 6.8 t ha^{-1}. According to *Ocokoljić et al. (1975)*, in the dry climate conditions of Serbian lowland region the average yield of birdsfoot trefoil hay of

11.9 t ha^{-1} could be achieved. By testing variability of productive features and quality of forage of selected birdsfoot trefoil genotypes, *Radović et al. (2007)* obtained the average hay yield of 11.34 t ha^{-1} of hay. The same authors point out that high results are the consequences of increased arability of soil on which testing was carried out. The same authors pointed out that high results are the consequence of soil arability on which the tests were carried out and that the yields of dry matter on the soil of lower quality, on which growing of birdsfoot trefoil is cost-effective, are considerably.

Birdsfoot trefoil can achieve good yields in seed production *(Petrović, 2011)*, so in combination with two cuts, considerable yield can be achieved for forage. The highest yields of forage in the densest crop establishment do not match the highest seed yield in three-year production, but they have the highest potential in combined use.

Conclusion

Birdsfoot trefoil is intended for seed production, and signficant amounts of biomass are achieved in addition to seed production. During three years trial the forage yield of birdsfoot trefoil was considerably changing depending on the tested factors. Production of forage was directly dependent on the age of crops, i.e. plant development, size of vegetation area and on the quantity of precipitation during the year. The highest hay yields were achieved in the third year of growing on the narrowest interrow spacing of 12.5 cm (6.56 t ha^{-1}) and at the highest seeding rate of 8 kg ha^{-1} (5.73 t ha^{-1}). Total production of hay in the three-year period of using the seed crop of birdsfoot trefoil ranged from 9.11 to 14.83 ha^{-1} depending on the interrrow spacing and from 10.74 to 13.2 ha^{-1} depending on the seeding rate. Birdsfoot trefoil in combined use (for seed production and forage) has the highest potential at higher density of crop establishment (8 kg ha^{-1} and 12.5 cm interrow spacing).

Uticaj gustine zasnivanja na prinos krme žutog zvezdana gajenog za kombinovano korišćenje

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Rezime

Žuti zvezdan za proizvodnju semena, sorte Bokor, ispitivan je u agroekološkim uslovima zapadne Srbije, u periodu 2007-2009. godine. Ispitivana je količina biomase koja se može dobiti gajenjem žutog zvezdana za seme, pri

zasnivanju na različitom međurednom rastojanju (12,5, 25 i 50 cm) i sa različitom normom semena (2, 4 i 8 kg ha^{-1}). Najveći prinosi sena ostvareni su u trećoj godini gajenja useva na najužem međurednom rastojanju od 12,5 cm (6,56 t ha^{-1}) i pri najvećoj količini semena od 8 kg ha^{-1} (5,73 t ha^{-1}). Ukupna produkcija sena u trogodišnjem periodu korišćenja semenskog useva žutog zvezdana bila je od 9,11 do 14,83 ha^{-1} u zavisnosti od međurednog rastojanja i od 10,74 do 13,20 ha^{-1} u zavisnosti od setvene norme. Žuti zvezdan u kombinovanom korišćenju (za seme i krmu) je imao najveći potencijal za prinos krme pri većoj gustini zasnivanja (8 kg ha^{-1} i 12,5 cm međuredno rastojanje).

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PUMPKINSEED (*Lepomis gibbosus* L. 1758) - UNWELCOME INHIBITANT OF ICHTHYOFAUNA IN THE RESERVOIR STREZEVO IN R. MACEDONIA

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Original scientific paper

Abstract: Pumpkinseed (*Lepomis gibbosus L*. 1758) represents an allochthonous (introduced) fish species in freshwater ecosystems of Europe. It has shown good adaptation to existing ecological conditions in the back and low flow rate waters. The analysis of some ecological characteristics of the samples, taken from the reservoir Strezevo in R. Macedonia, points to extremely good growth rate in this ecosystem. Despite markedly expressed fluctuations in the number of its population, pumpkinseed has occupied a corresponding ecological niche in the littoral part of habitat. However, due to a wide trophic spectrum and the possible competition for space and places for reproduction, pumpkinseed has become over-abundant, thereby deteriorating the ichthyofauna and state in reservoir Strezevo.

Key words: pumpkinseed, acclimatization, reservoir Strezevo, R. Macedonia.

Introduction

Fresh-water ichthyofauna is a dynamic component of aquatic ecosystems. In recent decades there were dramatic changes in qualitative and quantitative compound in fish stocks in waters in R. Macedonia. That is as a result of fish which are not permanent inhabitants in our waters. One of these kinds of fish is pumpkinseed (*Lepomis gibbosus*). It is an introduced kind which has naturalized a lot in the extended areas and is a serious problem for autochthonous ichthyofauna. It can be seen in its predator affinities, because for its nutrition it consumes roe and youth of other kinds. On the other hand, already existing predators do not consume pumpkinseed as their prey, so it has excellent conditions not only to survive, but to become over-abundant in newly conquered waters.

Pumpkinseed (*L.gibbosus*) is representative of family CENTRARCHIDAE and is autochthonous in North America. According to research of Wheeler (1983), fish of this family has increased in number and inhibited the confluence of

Mississippi river, at the same time inhabiting the Atlantic coast further to central parts of the USA. Largemouth bass (*Micropterus salmoides*) and Bluegill (*Lepomis macrochitus*) had exceptionally large distribution. These two kinds of fish are intentionally introduced in many reservoirs in the USA for sport fishing *(Moehl and Davies, 1993)*.

Representatives of family CENTRARCHIDAE have been successfully introduced in waters in Asia, Africa and Europe. In Europe there are four kinds, and in R. Macedonia only one - the pumpkinseed. All fish of this group are characterized with lively colors, hard, unbranched compacts in frontal part and soft branched in rear part of dorsal fin. Male fish, according to researches of *Garms and Borm (1981)* are significant and interesting because they make nest where they kept inseminated roe and spurge in the first days after being born.

Pumpkinseed is a small fish with maximum length of 20 cm. On the back it is olive - green colored, with dots which are orange on the stomach. There is dark spot on the operculum. In Europe it was introduced in 1887, as aquarium fish but it rapidly spreaded in open waters.

Pumpkinseed is fish with wide range of spreading: Western Europe, Pyrenees, England and other countries *(Povz, 1990; Elvira and Almodovar, 1994)*.

Materials and Methods

In this study was analyzed the pumpkinseed's (*Lepomis gibbosus*) population dense and good growth pace in the reservoir Strezevo in R. Macedonia. Population incerasement in number was studied in the period since 2004 to 2009 year, while the age and growth pace were analyzed on samples collected during 2007^{th}. For age determination were used squamas taken under dorsal fin and measurements of ventrolateral radius. The reconstruction of growth pace in previous period was calculated by logarithm function:

$$\log L = \log a + b \log S,$$

where L is total length, S is squama's size, a and b are calculated parameters *(Cugunova,1959)*.

Results and Discussion

According to lasting several years ichtiological researches in reservoir Strezevo in R. Macedonia it was determinate that fish settlement in reservoir Strezevo is made of basic ecologic groups related to their type of nutrition. There are three basic food chains in nutrition: plantofage (lake Ohrid bleak and silver carp), bentofage (linis, barbel, black barbel, carassius (crucian carp) and silver

carassius and carp) and ihtiofage (predators as trout, pike, catfish, eel and pumpkinseed).

Most common kinds in the reservoir are bleak, carassius, pumpkinseed and chub.

In order to perceive kind adaptation in existing conditions, we have made analyze on growth pace. The sample consisted of 28 units caught in the period spring - summer in 2007th year. The logorhythm function for the proportion between body length (L) and the size of squama diagonal radius (S) is:

$\log L = 0.3391 + 0{,}9297 \log S.$

The samples were from third group of age (age 2+) until sixth (5+) year. Measured body lengths were from 72mm to 121mm (Tab.1).

Table 1. Growth of body length pace for pumpkinseed *(Lepomis gibbosus*) in reservoir Strezevo

		Body length		Reconstruction of body length in mm				
Age	n	Average	Variation	l1	l2	l3	l4	l5
2+	11	81	72-86	45	71			
3+	8	91	83-100	47	67	83		
4+	6	104	96-109	44	64	78	95	
5+	3	112	106-121	50	69	78	101	110
		Average values (mm)		46	68	80	97	110
		Annual growth (mm)			22	12	17	13

The biggest length grow pace pumpkinseed achieves during the first year of life. In the third year there is a little regression in grow pace, due to period of maturity for sexual reproduction.

The references for pumpkinseed grow pace is deficient with data, so we have made comparison with grow pace of the population in reservoir Tapada Pequena, in south-east Portugal *(Godinho and Ferreira, 1996)*. Measured body length of pumpkinseed in that ecosystem was: age 1+ - 55,3 mm, 2+ - 73.3 mm, 3+ - 81.1 mm, 4+ - 90,1 mm and 5+ - 92,5 mm. If we do compare these results with the results from reservoir Strezevo, we can conclude that there is significantly more intensive growth of the population than in our ecosystem.

Pumpkinseed spawning is during the period April - May. The results showed that all fish were sexually matured at age from 2+ - 5+, which leads to conclusion that there is good naturalization to existing ecologic conditions. Maximum fertility for one fish at age of 3+ was 4357 eggs.

Pumpkinseed is fish that lives in stocks among plants along the coast. It is very aggressive and predatory, typical alerter. Except planktonic crustaceans (*Copepoda, Cladocera*) it eats insect *Diptera* larvaes, *Macrophytes* and youth of other fish. *(Vuković and Ivanović, 1971; Garmo and Borm, 1981; Godihno and Ferreira, 1996)*. Due to these nutrition characteristics, competition for living

spaces and reproduction places, intense number increase in population, pumpkinseed can threaten autochthonic ichthyofauna in our water ecosystems.

Conclusion

Pumpkinseed (*Lepomis gibbosus*) is allochthonous fish in fresh-water ecosystems in Europe. It has naturalized in existing ecological conditions in stagnant and low flow rate waters. The analysis of some ecological characteristics of the samples, taken from the reservoir Strezevo in R. Macedonia showed to extremely good growth pace in this ecosystem. Despite markedly expressed fluctuations in the number of its population, pumpkinseed has occupied a corresponding ecological niche in the littoral part of habitat. However, due to wide trophic spectrum and the possible competition for space and places for reproduction, pumpkinseed has become over-abundant, thereby deteriorating the ichthyofauna and state in reservoir Strezevo.

Sunčanica (*Lepomis gibbosus* L. 1758) - nepoželjan član akumulacije Streževo u Republici Makedoniji

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Rezime

Sunčanica (*Lepomis gibbosus L.* 1758) predstavlja alohtonu (novointrodukovanu) vrstu u slatkovodnim ekosistemima Evrope. Pokazala je dobru aklimatizaciju na postojeće ekološke uslove u stajaćim i slabo protočnim vodotocima. Analiza nekih ekoloških karakteristika kod primeraka iz akumulacija Streževo u R. Makedoniji, ukazuje na izuzetno dobar tempo dužinskog rasta u ovom jezerskom ekosistemu. Iako je populaciona brojnost podložna značajnim fluktuacijama, može se zaključiti da je vrsta zauzela odgovarajuće ekološko utočiste u priobalnom regionu staništa. Zbog širokog trofičkog spektra, kao i moguće kompeticije za prostor i mesta reprodukcije, omasovljenje sunčanice pogoršava stanje ihtiofaune ovog ekosistema.

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NUTRITIONAL VALUE OF THE GRAIN OF KUNITZ-FREE SOYBEAN CULTIVARS

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Abstract: Utilization of the raw beans for food and feed is prohibited by the presence of protease inhibitors. Breeding soybean cultivars for reduced amount of anti nutritional factors at Maize Research Institute Zemun Polje resulted in development of two new Kunitz-free cultivars – Lana and Laura. Feeding trials with pigs in different growth stages were conducted to compare the response of growing and finishing pigs to diets containing either raw or extruded standard and KTI-free (Kunitz trypsin inhibitor-free) soybean cultivars. The greatest improvement in growth performance was noted for the group fed extruded KTI-free soybean. This group achieved highest daily weight gain and gain per feed unit. Growth parameters for group of pigs fed raw soybean Kunitz-free cultivar were slightly reduced.

Key words: soybean, Kunitz trypsin inhibitor, feeding trials, pigs

Introduction

The protease inhibitors in soybean, Kunitz trypsin inhibitor (KTI) and the Bowman–Birk inhibitor (BBI) constitute at least 6% of the protein present in soybean seed *(Ryan, 1973)* and with lectin, represent the main anti nutritional factors of soybeans. Approximately, 80% of the trypsin inhibition is caused by KTI *(Brandon, 1993)*, which strongly inhibits trypsin, and therefore reduces protein digestibility and food intake. Beside that, KTI is responsible for hypersecretion of pancreatic enzymes, leading to demands of sulphur-containing amino acids and along side previous, resulting in growth depression in non-ruminant animals. Due to this, row soybean can not be used for animal feeding and needs to be heat-processed to eliminate thermo-labile anti nutritional factors. Heat processing inactivates anti-nutritional factors and modifies the structure of the proteins, making them more available for digestion. Despite the efficiency of thermal treatment to reduce protease inhibitors, residual inhibition (10-20%) is maintained *(Carvalho et al., 1998)*. Furthermore, excessive heat treatments (uncontrolled

temperature; long period of time) may decrease protein solubility and lower amino acid availability. Soybean lines with reduced protease inhibitor content could reduce or eliminate the need for expensive heat treatments and lessen the chance of lowering amino acid availability.

A part of the soybean breeding program at the Maize Research Institute »Zemun Polje« is aimed at developing the cultivars with reduced trypsin inhibitors content. As a result, two Kunitz-free (KTI free) varieties Lana and Laura, were released. TI content in new cultivars was about 50% reduced as compared with the conventional cultivars (standard grain type). Numerous studies investigated the effect of soybean variety and processing on growth performance of pigs *(Cook et al., 1988; Palacios et al., 2004)*. They demonstrated that the inclusion of raw Kunitz-free soybean in diet was beneficial in terms of better growth performance compared with conventional cultivars, but still inferior to the growth performance obtained by soybean meal, where the KTI and other anti nutritional factors are inactivated by heating. The results obtained by *Tagliapietra et al. (2007)*, suggest that raw soybean can be usefully included in the diets of finishing pigs (80 to 170 kg LW) up to 10% of the complete feed without negative effects on growth performance and health status.

Regarding previous issues, present study was carried out to estimate the nutritional value of new cultivars in feeding trials with pigs and determine whether pigs in different stages of development could be successfully fed raw Kunitz-free soybean cultivars.

Materials and Methods

During 2008 year, an experiment with pigs was conducted at the „Stari Tamiš“ farm, with two main goals. First, to evaluate the effect of raw soybean on growth performance and health of growing/fattening pigs and second, to determine differences in weight gain of animals fed the mixtures formulated to contain: 20% grits made by extrusion of standard grain quality soybean (Mixture 1), 20% grits of extruded grain of Kunitz-free soybean cultivars (Mixture 2), 20% row Kunitz-free soybean cultivars (Mixture 3).

Table 1. Number of piglets and initial live weight of the pigs in the experiment

	Mixture 1 (extruded standard cult.)	Mixture 2 (extruded KTI-free)	Mixture 3 (raw KTI-free)
Number of animals	14	14	14
Initial weight	23.00	23.20	22.90
Growing weight (Stage I)	56.35	55.60	52.12
Finishing weight (Stage II)	110.35	116.60	103.73

The sample consisted of 14 animals per each mixture, with initial body weight of 23 kg. Individual daily weight gain (DWG) of pigs was recorded in two stages: Stage 1 (body weight of feeders 56.35 kg, 55.60 kg and 52.12 kg, for the groups fed Mixture 1, Mixture 2 and Mixture 3, respectively) and Stage 2, where the finishing body weight was 110.35 kg, 116.60 kg and 103.73 kg for groups fed Mixture 1, Mixture 2 and Mixture 3, respectively (Table 1). The amount of feed distributed to each group was daily recorded.

Results and Discussion

The growth performance data from the pig experiment showed that the best average daily weight gain (DWG) of pigs was achieved for the group fed mixture with extruded KTI-free soybean cultivar (Mixture 2) in Stage 2 (Table 2). This group performed the best overall growth rates (Index=106.87%). Feeding raw KTI-free soybean (Mixture 3) to pigs depressed daily weight gain by 14.29% as compared with the average DWG achieved by pigs fed extruded KTI-free cultivar (Mixture 2). The growth depression could be explained by other heat-labile anti-nutritional factors present in raw soybeans – Bowman-Birk trypsin inhibitor and lectin. Previous results suggest that simple removal of Kunitz inhibitor without any intervention upon Bowman-Birk inhibitor, although allowing a reduction of processing costs *(Friedman et al., 1991)* does not appear to solve a problem of direct livestock nutrition.

The index of average DWG was by 7.42% higher for pigs fed mixture containing extruded standard cultivar than for those fed mixture with raw KTI-free soybean. This suggests that Kunitz-free varieties may present good solution for small farms with direct feed production and raising livestock, especially for the regions where the processing industry is not developed.

Table 2. Average daily weight gain (g) of groups of pigs fed different mixtures

	Mixture 1 (extruded standard cult.)	Mixture 2 (extruded KTI-free)	Mixture 3 (raw KTI-free)
1 Stage	641	623	562
Index %	100.00	97.19	87.67
2 Stage	794	897	759
Index %	100.00	112.97	95.59
Average	728	778	674
Index %	100.00	106.87	92.58

Furthermore, the average DWG of pigs fed Mixtures 1, 2 and 3 differed in various stages of the pig's development (Stage 1 and Stage 2). The average daily weight gain (DWG) achieved by pigs fed raw soybean in Stage 2 slightly depressed by only 4.41% as compared with average DWG of pigs in same stage fed extruded standard soybean. Average DWG of pigs in early stage of development (Stage 1) showed a strong depression by 12.33% for group fed Mixture 3, as compared to those fed Mixture 1. These results suggest that the effect of Kunitz trypsin inhibitor depends on age, and might be more harmful for young animals than for adults, which is in accordance with the results obtained by *Baker (2000)*. On the contrary, *Walker et al. (1987)* reported that replacing heated soybean meal with raw soybeans similarly reduced performance of both growing and finishing pigs.

The average daily feed intake varied less than 3% among dietary treatments. The absence of significant variations of this parameter was reported also in the studies of *Cook et al. (1988)* and *Tagliapietra et al. (2007)* (Table 3).

Table 3. Average daily feed intake (kg)

	Mixture 1 (extruded standard cult.)	Mixture 2 (extruded KTI-free)	Mixture 3 (raw KTI-free)
Stage I	1.63	1.65	1.64
Index %	100.00	101.23	100.61
Stage II	2.76	2.82	2.80
Index %	100.00	102.17	101.45
Average	2.23	2.28	2.29
Index %	100.00	102.24	102.69

The values of food conversion ratio observed for three groups of pigs showed that the highest average conversion value (3.40) was obtained by group of pigs fed mixture containing raw Kunitz-free soybean cultivar (Mixture 3) (Table 4).

Table 4. Food conversion ratio

	Mixture 1 (extruded standard cult.)	Mixture 2 (extruded KTI-free)	Mixture 3 (raw KTI-free)
Stage I	2.54	2.65	2.92
Index %	100.00	104.33	114.96
Stage II	3.48	3.14	3.69
Index %	100.00	90.23	106.03
Average	3.06	2.93	3.40
Index %	100.00	95.75	111.11

Average food conversion calculated for the group fed Mixture 2 (extruded Kunitz-free soybean) showed the lowest value (2.93). This group of pigs consumed in average 15.36% less feed for the unit of weight gain compared to the group fed Mixture 3.

The lower level of performance (DWG and G:F) of the group of pigs fed raw Kunitz-free soybean showed that the nutritional value of raw soybean, although higher than that of the standard variety, is still to low to sustain normal growth and development of animals. Nevertheless, KTI free soybean cultivars can offer nutritional advantages, since they need a shorter heating time for inactivation of trypsin inhibitors *(Friedman et al., 1991)*. Furthermore, such varieties might be processed more economically into human foods as well.

Conclusion

Although the nutritional value of raw Kunitz trypsin inhibitor-free soybean varieties is diminished by other heat-labile factors present in grain, such varieties might be processed more economically with shorter heating time and lower temperature, or utilized in extensive farming systems.

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Nutritivna vrednost zrna sorti soje bez Kunitz-tripsin inhibitora u zrnu

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Rezime

Upotreba zrnastih mahunarki u sirovom stanju u ishrani ljudi i domaćih životinja ograničena je prisustvom inhibitora proteaza u zrnu. Oplemenjivanje soje na smanjen sadržaj antihranljivih materija u Institutu za kukuruz Zemun Polje rezultiralo je stvaranjem dve nove sorte soje bez Kunitz tripsin inhibitora – Lana i Laura. U cilju upoređivanja efekata ishrane koja sadrži sirovo ili ekstrudirano zrno soje standardnog kvaliteta zrna i soje bez Kunitz tripsin inhibitora, postavljeni su ishranbeni ogledi sa svinjama u različitim fazama tova. Najveći napredak u pogledu brzine porasta je ostvaren kod grupe hranjene ekstrudiranim zrnom soje

bez Kunitz tripsin inhibitora. Ova grupa postigla je najveće vrednosti prosečnog dnevnog porasta i porasta po jedinici konzumiranog hraniva. Parametri rasta kod grupe svinja hranjene sirovim zrnom soje bez Kunitz tripsin inhibitora bili su blago umanjeni u odnosu na ostale ispitivane grupe.

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SEASONAL CHANGES OF PROTEINS, STRUCTURAL CARBOHYDRATES, FATS AND MINERALS IN HERBAGE DRY MATTER OF RED CLOVER (*Trifolium pratense* L.)

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Abstract: Red clover (*Trifolium pratense* L.) is the second most important perennial forage legume, after alfalfa. It is a significant source of proteins, which are essential in the diet of domestic animals, especially ruminants. The objective of this study was to determine the most important characteristics of dry matter quality (crude proteins, structural carbohydrates, fats and minerals) in three varieties of red clover (K-17, Kolubara and Una). The characteristics were monitored, per individual cuts, during one production cycle of red clover, i.e., in the period 2004-2006. Highest contents of crude protein in all three test years were obtained in the second cut. Significantly increased contents of crude proteins (18.32%) and potassium (K-2.53%) were found in the red clover cultivar Una developed in Novi Sad. Significantly increased values of both cellulose fiber fractions (NDF, ADF) were registered in the second year of growing. The highest content of minerals was determined at the beginning of the growing season in the first and second year (8.68% and 8.52%, respectively). Here it should be mentioned that the contents of basic biogenous elements, phosphorus and potassium, were within the expected optimum range.

Key words: red clover (*Trifolium pratense* L), forage quality, cultivar, cut, seasonal changes

Introduction

Red clover (*Trifolium pratense* L.) is the second most important perennial forage legume, after alfalfa. As fodder, it is used both fresh and processed (hay, haylage, flour, silage). Red clover exceeds corn and oat grains in the content of essential amino acids (cystine, tryptophan, leucine). Red clover foliage is

particularly rich in proteins. Protein content reaches about 25% at the stage of budding, which makes red clover leaves an important ingredient of quality forage *(Vasiljevic et al., 2009)*. The high content of soluble carbohydrates makes it a good energy feed, better than alfalfa or Italian ryegrass. Red clover contains large amounts of provitamin A and vitamins C, D, E, K, B_1, B_2 and B_3. It is also rich in minerals *(Marković et al., 2007)*.

The quality of red clover forage depends primarily on the stage of development and environmental conditions. *Taylor and Quesenberry (1996)* claimed that the content of crude proteins and *in vitro* dry matter digestibility are the two most reliable quality characteristics. Their value decreases with age, as a result of a decreasing portion of leaves in relation to stems and the process of lignification, which are typical for all perennial legumes. The decline in digestibility after budding is associated with increased contents of lignin and structural polysaccharides.

Stage of development, i.e., plant age, affects considerably the chemical composition and forage quality of red clover *(Ignjatović et al., 2001; Marković et al., 2007; Belanger, 2010)*. In early spring, young red clover plants have a high proportion of leaves, high contents of moisture, proteins and minerals and a low content of fibers. During growing season, under the effect of longer days and higher temperatures, the aging plants undergo morphological changes: leaf growth becomes slower, the stem increases in length and the proportion of dry matter increases. On the other hand, forage quality decreases drastically, especially digestibility and the contents of proteins and minerals. The optimum time for red clover cutting, in terms of both, forage quality and total yield, is the stage when 20-25% flowers are in bloom *(Fairey, 1988; Wiersma et al., 1998)*. At that stage, dry matter digestibility ranges from 65 to 70%.

Also, the stress caused by unfavorable environment as well as by seasonal changes may cause a significant decline in forage quality of red clover, as a consequence of altered leaf to stem proportion (in favor of the stem) and accelerated aging *(Taylor and Qusenberry, 1996)*. However, the research on red clover conducted by *Buxton et al.* (1985) showed that the aging does not cause a drastic drop in quality as is the case with some other perennial legumes (such as alfalfa and yellow trefoil). This is due to a higher portion of leaves and lower contents of cell wall and lignin *(Buxton and Hornstein, 1986)*.

The objective of this study was to determine, in the course of a three-year production cycle (2004-2006), the most important characteristics of dry matter quality (crude proteins, structural carbohydrates, fats and minerals) in three varieties (K-17, Kolubara and Una), which dominate the commercial red clover production in the Republic of Serbia.

Materials and Methods

The red clover varietal trial was established in spring 2004 at the experiment field at Rimski Šančevi, on the chernozem soil. The trial was organized in a randomized block design with five replications. The experimental unit area was 5 m^2, the row spacing was 20 cm, and the seeding rate was 15 kg ha^{-1}. Two cuts were performed in the first year, the first one in the second half of July (21 July 2004), at the stage of full bloom, and the second in the first half of September (11 September 2004). Four cuts were performed in the second year of the trial: 13 May, 23 June, 1 August and 6 October 2005, all in the early blooming stage. Two cuts were performed in the third year, on 18 May and 27 June 2006. The most important quality characteristics of red clover herbage (crude proteins, structural carbohydrates, fats, minerals, macroelements P, Ca, K and Na) were determined by conventional methods performed in the laboratory of Department for Animal Feed Analysis of Department for Animal Husbandry, Faculty of Agriculture in Novi Sad.

Results of the three-year trial were analyzed by the analysis of variance and tested by the LSD test.

Meteorological conditions. Novi Sad and its surroundings are located in a zone of the temperate continental climate. The long-term (1948-1993) average precipitation for the vegetation period is 338 mm and average temperature for the same period is 17.8^{o}C (Table1).

Table 1. Rainfall and mean monthly temperatures per growing season (2004-2006) and long-term averages (1948- 1993)

Month	Rainfall (mm)				Temperature (^{o}C)			
	2004	2005	2006	1948-1993	2004	2005	2006	1948-1993
IV	112	31	65	48	12,5	12,0	13,0	11,4
V	89	37	72	58	15,2	17,0	16,0	16,5
VI	97	138	104	83	19,8	19,0	20,0	19,7
VII	63	123	32	61	22,0	21,0	23,0	21,4
VIII	39	135	123	52	21,7	20,0	20,0	20,9
IX	42	66	23	36	16,3	18,0	19,0	17,0
Total	442	530	419	338				
Average					17,9	17,8	18,5	17,8

In the three years of the trial (2004-2006), the rainfall sums per vegetation period were significantly above the long-term average. The monthly rainfall was unevenly distributed. The significantly higher rainfall than the long-term average recorded in April 2004 (112 mm) was quite favorable for successful red clover establishment. Maximum rainfall in the second year of the trial was reached in the summer months (June, July and August). It was nearly 2 to 2.5 times higher than

the long-term average for that period, and it positively affected the quality of forage from the second and third cuts (increased crude proteins content). The rainfall sums recorded in June and August 2006 (104 mm and 123 mm, respectively) were much higher than the long-term average values.

Considering the mean monthly temperatures for the vegetation periods of 2004-2005, it can be noted that there was no major departure from the long-term averages. The minor deviations from the long-term average values recorded in April, July and August 2006 increased the average temperature for the vegetation period by about 1°C.

Results and Discussion

Analyzing the content of the studied quality characteristics in three red clover cultivars, statistically significant differences were found between the cuts (Table 3) but there was no significant difference between the cultivars (Table 2). Still, Una, the red clover cultivar developed in Novi Sad, had somewhat increased contents of crude proteins and potassium (K) (Table 2).

Table 2. Variation among red clover cultivars in crude proteins, structural carbohydrates, fats and minerals during 2004-2006

Cultivar	Crude proteins (%)	Structural carbohydrates (%)		Crude fats (%)	Ashes (%)	Minerals (%)			
		NDF	ADF			Ca	P	Na	K
K-17	17.59	39.41	30.61	2.04	7.89	1.90	0.29	0.10	2.29
Kolubara	17.96	38.63	29.17	1.99	8.15	1.89	0.30	0.07	2.45
Una	18.32	36.97	30.11	1.97	8.18	1.80	0.30	0.08	2.53
Average	17.95	38.34	29.96	2.00	8.07	1.86	0.30	0.08	2.42
LSD 0.05	0.72	3.28	1.52	0.29	0.58	0.14	0.03	0.03	0.20
0.01	0.99	4.55	2.11	0.41	0.81	0.20	0.05	0.05	0.28

In the three years of research, variation of crude proteins content in red clover dry matter was significant among the different cuts (Table 3). The highest content of crude proteins was found in the second cut in all three years, indicating a seasonal change in quality. The increased protein content in dry matter from the second indicated that this part of growing season had favorable agro-ecological conditions for leaf formation.

Table 3. Seasonal variation in crude proteins, structural carbohydrates, fats and minerals in red clover cultivars during 2004-2006

Date of cutting	Crude proteins (%)	Structural carbohydrates (%)		Crude fats (%)	Ashes (%)	Minerals (%)			
		NDF	ADF			Ca	P	Na	K
21.07.2004	15.81	35.6	30.2	2.13	8.68	1.98	0.25	0.11	2.71
11.09.2004	17.99	33.4	22.9	2.55	8.11	2.07	0.24	0.05	2.08
13.05.2005	16.82	35.6	29.7	2.13	8.48	1.73	0.30	0.11	2.56
23.06.2005	19.48	42.0	30.5	1.76	8.52	2.00	0.31	0.08	2.72
01.08.2005	18.39	38.5	32.5	2.48	7.85	1.81	0.32	0.07	2.51
06.10.2005	19.40	45.5	33.8	1.68	8.21	1.66	0.37	0.09	2.54
18.05.2006	17.92	37.9	28.6	1.72	7.45	1.97	0.24	0.07	1.93
27.06.2006	18.23	38.1	31.6	1.54	7.31	1.68	0.36	0.11	2.35
Average	17.96	38.3	29.97	2.00	8.08	1.86	0.30	0.09	2.43
LSD 0.01	1.17	5.36	2.49	0.48	0.95	0.23	0.06	0.06	0.33
0.05	1.63	7.44	3.45	0.66	1.32	0.33	0.08	0.08	0.46

Knowledge of changes in quality of red clover forage during growing season is important when preparing meals for ruminants, to ensure a satisfactory relationship between structural and unstructured carbohydrates *(Grubić and Adamović, 2003)*. Considering the contents of neutral and acid detergent fibers highest values were obtained in the second year of growing (Table 3), when red clover had the highest total biomass production *(Vasiljević et al., 2010)*.

Low but, in terms of ruminant feeding, optimum values of NDF and ADF (33.4% and 22.9%, respectively) were obtained in the fall cut in the year of red clover establishment (Table 3).

Studying the content of individual fractions of structural carbohydrates NDF, ADF, hemicellulose) in five red clover cultivars grown in the period 2004-2006, *Vasiljević et al. (2008)* found statistically significant differences between the cuts (Table 4), while no difference was found between the cultivars (Table 5). The contents of both fractions of cellulose fiber (NDF and ADF) was increased in summer, due to high temperatures and accelerated aging (development) of plants.

Significantly high contents of fats were registered or at the end or in the later part of growing season in the first and second year of growing (Table 2). In contrast to the seasonal variation of fats content in dry matter of red clover, there were no significant differences between the cultivars (Table 1).

Red clover is a rich source of minerals which are essential in the diet of ruminants. The content of minerals is high in early stages of red clover growth, i.e., in the leaves. This in turn indicates the importance of preserving leaves during processing, storage and use of red clover hay. Highest contents of minerals were recorded at the beginning of the growing seasons (first and second cutting) in 2004 and 2005 (Table 3).

Studying the quality characteristics of dry matter at different stages of development of red clover cultivars, *Vasiljevic et al. (2005)* found that there was no difference in the content of minerals at the stages of budding and early bloom, while the average ash content in the two stages was 8.9%. A somewhat lower value of this characteristic was found at the stage of full bloom (7.8%). *Dinić et al. (1990, 1994)* reported the ash content at the stage of early bloom in red clover of 10.3%.

In addition to nitrogen (N), other important biogenous elements in the diet of ruminants are phosphorus (P), potassium (K) and calcium (Ca). The effect of seasonal variations on the contents of these macroelements in red clover dry matter was more important than the effect of genotype. The phosphorus content per cut ranged from 0.23 to 0.37%, the potassium content from 1.93 to 2.72% (Table 3). Highest contents of calcium (Ca), similarly to the crude proteins content, were determined in dry matter from the second cuts in 2004 and 2005.

Fairey (1988) stated that the phosphorus content in red clover dry matter ranges from 0.2 to 0.4%, if the yield is not limited by phosphorus deficiency. Thus, to achieve a dry matter yield of 10,000 kg ha^{-1} it is necessary to provide between 20 and 40 kg ha^{-1} phosphorus (P) per year. The same author also reported that there is about 1.8% of K in red clover dry matter, so, to achieve a dry matter yield of 10,000 kg ha^{-1} it is necessary to provide red clovers with 180 kg ha^{-1} of potassium (K) per year.

Conclusion

The three-year research showed that specific seasonal changes occurred in the most important quality characteristics of dry matter (forage) of the three red clover cultivars (K-17, Kolubara, Una) that dominate the commercial production in the Republic of Serbia.

Highest contents of crude proteins and calcium (Ca) in red clover dry matter were determined in the second cuts in 2004 and 2005. These high values indicated that this part of growing season has favorable agroecological conditions for leaf formation.

Significantly high contents of crude proteins (18.32%) and potassium (2.53%) were found in the cultivar Una, which had been developed in Novi Sad.

Highest values of both fractions of cellulose fiber (NDF and ADF) were determined in the second year of growing, when red clover also had the largest total biomass production.

Significantly high contents of fats were recorded at the end or in the later part of growing season in the first and second year of growing (2.55% and 2.48%, respectively).

Highest contents of minerals were found at the beginning of the first and second growing season. It should be noted here that the contents of basic biogenous elements, phosphorus and potassium, were within the expected limits.

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Sezonske promene sadržaja proteina, strukturnih ugljenih hidrata, masti, mineralnih materija u suvoj materiji crvene deteline (*Trifolium pratense* L.)

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Rezime

Crvena detelina *(Trifolium pratense* L.) je druga po značaju višegodišnja krmna leguminoza. Slično lucerki značajan je izvor proteina, neophodnih u ishrani domaćih životinja, a naročito preživara. Cilj rada je bio da se tokom jednog proizvodnog ciklusa crvene deteline, u periodu 2004-2006. godine po otkosima utvrde najvažniji parametri kvaliteta suve materije: sadržaj sirovih proteina, strukturnih ugljenih hidrata, masti i mineralnih materija kod tri domaće sorte crvene deteline: K-17, Kolubara i Una. Najveći sadržaj sirovih proteina u sve tri godine ispitivanja je utvrđen u drugom otkosu. Značajno veći sadržaj sirovih proteina (18,32 %) i kalijuma (K-2,53 %) je utvrđen kod NS-sorte crvene deteline Une. Statistički značajno veće vrednosti obe frakcije celuloznih vlakana (NDF, ADF) su utvrđene u drugoj godini života crvene deteline. Najveći sadržaj mineralnih materija je utvrđen početkom vegetacije prve (8,68 %) i druge godine proizvodnog ciklusa (8,52 %) crvene deteline, pri čemu treba istaći da se sadržaj osnovnih biogenih elementa: fosfora i kalijuma nalazio u očekivanim optimalnim granicama.

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TRENDS IN LEGUMES ENSILAGING

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Review paper

Abstract: Modern trends in legumes ensilaging technology are based on the knowledge of biomass from the aspect of suitability for ensilaging, wilting, addition of carbohydrate feed, use of biological additives, etc. Today, the experiments are conducted, worldwide, with inoculates, which, in addition to homofermentative, also contain heterofermentative lactic acid bacteria. Products of such inoculants contribute to the increase of aerobic stability of silages so their implementation is good for all types of silage. In addition to the usage of those additions, modern technology of silage is based on the maximum mechanization of the ensilaging process, as well as preparing the silage in the form of roto-bales and silo tubes (most inexpensive way of conservation) as well as permanent facilities.

Key words: legumes, silage, carbohydrates, buffer capacity, wilting, carbohydrate feed, inoculants

Introduction

The general characteristic of livestock nutrition in the lowland and mountain area of Serbia is unfavorable ratio of nutritive substances, or the lack of protein feed. Excess energy in the feed is the result of the presence of maize grain, silage and the corn plant parts. Solution to this problem is to increase the proportion of protein feed in meals. The concentrated protein feed (pellets, oilseed cakes, etc.), are mainly imported and their price is high. A solution to the above problem is to increase proportion of the quality of the bulk food made of the legumes.

Of perennial legumes used for the production of the bulk feed in Serbia, the most important are: alfalfa, red and white clover, bird's-foot trefoil and lupine. Of annual legumes the most important are vetch and forage pea, and less significant are soy, lupine, forage fava beans, etc.

The importance of legumes for the forage feed production

High nutritious value of voluminous mass of legumes is underused. The lesser part of total amount of legumes is used as the green mass, while the most part is conserved using different techniques. Conserving of the legumes, as well as other feed, is the inevitable need, primarily because of providing of food during the annual period of vegetation hibernation *(Djordjevic and Dinic, 2003)*. Livestock feed with legumes, in relation to grass feed, provides better utilization of production potential of livestock. Factors that contribute to the superiority of legumes in relation to the grass are: the animals consume larger quantities; decomposition of consumed food and the exploitation of the nutritional substances are more efficient and it contains more specific biological elements *(Marten, 1984)*. One of the most important characteristic of legumes is the ability to use of nitrogen from the atmosphere and the preservation of ecosystems. Alfalfa is the most important forage plant in Serbia. It provides high and stable yield, in the experimental conditions, of 15-20 t of dry matter and about 3.000 kg of crude protein after the first year *(Mejakic et al., 1997)*. In addition to a high ratio, alfalfa protein contains a large amount of carotene, xantophyll and calcium and is first class of forage feed for all livestock. In favor of this are the studies conducted by *Sredojevic et al. (1991)*, in which carotenoide-protein concentrate are produced from the alfalfa, which in the dry matter contains 57% of crude protein, crude cellulose 2.10%, 10.57% of crude fat, 35.35% of essential amino acids, 1052 mg kg^{-1} of xantophyll and 547 mg kg^{-1} of β-carotene.

Red clover is the second important forage culture of the group of perennial legumes. Behind it is white clover, bird's foot trefoil, lupine and others. Quality, as well as in alfalfa, depends on the phase of plant development. Content of crude protein in the dry matter of red clover is 180-200 g kg^{-1} DM, crude cellulose from 210-257 g kg^{-1} DM, NFE 430-440 g kg^{-1} DM, Ca 13-15g kg^{-1} DM and P 3.3g kg^{-1} DM *(Dinic et al., 1994a; Djukic et al., 2008)*.

In terms of the content of nutrient substances for livestock feed, biomass of white clover is one of the highest forage quality. It has the high content of protein and minerals and low content of crude cellulose *(Dinic et al., 1994a)*. It is the one of the forage plants with the most uniform nutritional substances content during the vegetation period, meaning there is the least quality oscillations. The chemical composition of red and white clover and the silage suitability are presented in the Table 1.

Tabele 1. Chemical composition and buffer capacity of red clover, white clover and maize meal, g kg^{-1} DM *(Dinic et al., 1994a)*

Parameters	Red clover (a1)		White clover (a2)		Maize meal
	Non wilted	Wilted	Non wilted	Wilted	
Dry matter	204.0	320.0	161.3	281.3	870.0
Organic matter	897.4	899.1	880.3	894.0	890.4
Ash	102.6	100.9	119.7	106.0	19.6
Crude proteins	180.4	192.5	257.3	240.8	101.7
Crude fiber	257.1	247.3	232.4	212.1	26.4
Lipids	21.6	28.7	31.1	26.3	48.3
NFE	438.3	430.6	359.5	411.4	804.0
Ca	14.3	12.9	13.0	12.6	0.2
P	3.2	3.3	4.2	3.9	3.4
Sugar (S)	106	124	87	95	44
Buffer capacity (BC)	60	58	72	50	16
S/BC	1.77	2.14	1.21	1.90	2.75

Of perennial legumes in Serbia, mostly forage pea and vetch are used for the production of the bulk animal feed, and rarely or very rarely soy, fava bean and lupine. Annual legumes are very important group of forage crops, because they provide high yield of green mass (30-50 t ha^{-1} green mass) of the excellent quality. In the pure crop, protein content was 18-22%, 22-26% of cellulose and 33-40% NFE in the dry matter *(Miskovic, 1986)*. They can be cultivated in winter, spring or summer sowing, both as a single crop or combined with grain cultures. They, also, fixate nitrogen as the other legumes.

Annual legumes, due to the tendency to lie down, are cultivated the combination with grain crops (oats, rye, wheat, etc.). The quality of biomass, in this case, depends on the proportion of legumes and grain crops. However, in some studies, annual legumes are cultivated in combination with grasses and perennial legumes. In the studies of *Latre et al. (2008)* there were following combinations: barley + pea, pea + perennial ryegrass + white clover, forage fava beans + spring wheat and pea + alfalfa. When there are favorable conditions or where irrigation is possible, there is a possibility of cultivating of annual legumes and grain crops in summer sowing. *Dinic et al. (1999)* ensilaged crops of summer sowing: maize + soybean, maize + forage fava beans, sorghum + soybean and sorghum + forage fava beans in different weight ratio, in percents, (100:0, 75:25, 50:50, 25:75 and 0: 100), where the initial material was of the quality presented in Table 2.

Table 2. Chemical composition of green mass, g kg^{-1} DM *(Dinic i sar., 1999)*

Sample	CP	CF	Ash	Ca	P
Zea mays	88.7	252.1	65.0	2.8	3.3
Sorghum	84.4	294.6	92.9	3.6	3.4
Soybean	207.2	261.0	89.1	8.6	4.0
Vicia faba	165.9	366.5	82.9	3.8	4.9
LSD 0,05	10.23	25.66	4.69	1.00	0.71
LSD 0,01	15.50	38.88	7.10	1.56	1.07

Suitability of legumes for ensilaging

Biomass conservation, especially the biomass of perennial legumes, is followed by certain losses in dry matter. Due to that, the best is to use the green biomass (pasture). But there is no vegetation in the course of the year, and some legumes have anti-nutritive properties, and can cause certain problems in animal nutrition (bloating, etc.). So, it is necessary to conserve biomass. Hay and silage are the main forms of conserved bulk feed. The hay quality is influenced by weather conditions, so hay is less prepared and used. Its place is taken by silage. Hay is, above all, expensive feed, but the minimum amounts are necessary for normal functioning of the complex digestive system of ruminants. In many countries, there is trend to prepare and use of large amounts of silage compared to hay *(Wilkinson and Toivonen, 2003)*.

Ensilaging is the process of conservation of plant biomass and plant by-products by lactic acid. Lactic acid is the product of the natural microflora or added (inoculated) selected lactic acid bacteria strains. Suitability of plants for ensilaging can be precisely determined based on the sugar (s) content and on the buffer capacity (BC) *(Dinic et al., 1998)*.

High buffer capacity of legumes (a consequence of the high percentage of protein and minerals - calcium) and small amounts of fermentable carbohydrates are the main limiting factors for the application of silage technology for legumes on the large scale *(Dinic et al., 1998)*. Therefore, since the thirties of the twentieth century, many experiments were performed around the world. The task is to find ways for the successful alfalfa red clover, pea, vetch, soybean, and etc. conservation by ensilaging *(Djordjevic and Dinic, 2003)*. During the development of technology of silage the results of numerous experiments have been successfully used in practice (chemical preservatives based on organic acids, carbohydrate additives, wilting and biological products). Today, in Europe and worldwide, biological products and organic acids are mostly used. The main goal of the modern technologies is the making of silage with higher quality, with the smaller losses of dry matter, the maximum aerobic stability and nutritive value.

The wilting

The amount of dry matter in silo mass is one of the most important factors for the level of losses of dry matter and directing the fermentation process in silo mass, especially when it comes to silo mass that is rich in protein and minerals *(Dinic, 1990; Dinic et al,. 1997)*. Wilting, or short-term drying, is certainly the least expensive solution for a successful ensilaging of legumes and mixtures of legumes and grain crops. Effective conservation of wilted crops is explained by ability of lactic acid bacteria to be active in the area with increased osmotic pressure, while most other anaerobic microorganisms can not compete with them. Positive experiences in using this technology are demonstrated experimentally and in local research *(Dinic, 1990; Djordjevic, 1995)* and are largely applied in practice on our farms *(Djordjevic and Dinic, 2003)*.

There were a lot of studies on the subject of silo mass wilting, but the most complete researches were done under the direction of *Zimmer and Wilkins's (1984)*. The general conclusions of their researches were that there were smaller losses of dry matter in the field in non wilted silo mass (2.5:8.6%), significantly higher in the silo in the silage of non wilted silo mass with preserving agent in relation to the wilted silo mass without preserving agent (16.1:8.5%). Silages were generally well kept in the two treatments. The preserving agents in silage from non wilted silo mass were based on formic acid, and the wilted silo mass had a 30-40% of dry matter. Wilting is important due to restraining impact on the level of proteolysis in the legume silages *(Djordjevic et al., 2004)*. However, the possibility of wilting depends on weather conditions, and can not be always done. Also, wilting requires further engagement of mechanization and manpower for the collection of forage mass from the ground, which can easily lead to the contamination with dirt, sand and other substances. Only the biomasses of annual legumes + grain crops in the phase of the beginning of pod formation, with dry matter content about 300 g kg^{-1} can be successfully ensilaged without wilting, or with the direct ensilaging *(Dinic et al., 2008)*.

Mixing with the easily ensilaged crops and the addition of carbohydrate feed

The mixing with the crops that are easy for the ensilaging is usually done in the early fall, at the time of the last alfalfa cut, as well as silage maize or sorghum from the main or additional sowing *(Dinic et al., 1988, 1995)*. In addition, it is possible to combine alfalfa with grain crops, but in this cases is used mainly the second alfalfa cut *(Djordjevic and Dinic, 2003)*. In such combinations, the

grain crops provide the required amount of carbohydrates, and legumes enrich ensilaged mass with protein. In the studies by our authors, it is found that the maximum share of alfalfa in the combined silages with maize is 50: 50% *(Dinic et al., 1988)*. On the practical side, the ratio of maize and alfalfa in the mixture for ensilaging depends first of the available amount of alfalfa.

In order to provide the necessary amount of carbohydrates for lactic fermentation it is practiced to add carbohydrate feed such as ground maize (5-10%), molasses (1%), dry chopped beet (5-10%) and others *(Koljajic et al., 1997)*.

The use of chemical preserving agents

The chemical conservation of crops is based on the inhibition of the plant enzymes by reducing the pH values below 4.5. Today, a small number of chemical preserving agents, mostly organic acids (formic and propionic) and their salts, are being used worldwide. One of the main reasons for this is the aspiration to produce food, both for human and livestock nutrition, as natural as possible, and to obtain the healthier products. In the last decades, in our country, numerous experiments with the use of formic acid were conducted. The effects of formic acid were compared with chemical preservative agents of mineral acids and their salts, carbohydrate additives or bacterial inoculates *(Dinic et al., 1994b, 1996, 1997; Djordjevic et al., 2003)*. Despite very good results, these kinds of additives are not used in our country because of high prices.

The use of biological additives

The advantage of biological additives in relation to the chemical preserving agents is primarily in that they do not leave residua and do not negatively affect the health of livestock and on the quality of their products. Due to this, they increasingly push out the chemical preserving agents, regardless of their lower efficiency. The experiments conducted in our country confirmed the positive influence of the biological preparations *(Djordjevic, 1995; Djordjevic et al., 2000)*, which are now applied in practice in our large farms *(Djordjevic and Dinic, 2003)*. The greatest effects of bacterial inoculation are expressed in the feed which are difficult to ensilage, especially legumes. By using the homofermentative bacteria cultures the present soluble carbohydrates, which are at the border sugar minimum, are being exploited.

The original products based on lactic acid bacteria contained the homofermentative microorganisms in monoculture or in the combination of several species. The majority of the experiments proved that the combination of different bacteria is more flexible in regard to the choice of the nutritional substances, temperature intervals and other conditions, meaning they complete various aspects

of their activities, in relation to the monoculture. Modern biological preparations contain *Lactobacillus plantarum* and other *Lactobacillus* species, in combination with *Enterococcus*, *Lactococcus* and *Pediococcus* species. In the next phase of development of this technology there is a trend of combining the lactic fermentation bacteria with celulolitic enzymes. By using these products, the intensification achieved of lactic acid fermentation of homofermentative type is achieved. Also, the decomposition of the different fractions of fibers, and at the same time increasing of the content of fermentable carbohydrates, is achieved. Recently, a great attention is paid to the increasing of aerobic stability of silages, especially of maize silages, but, also, of other forage species, due to lactic acid being the main product of fermentation of sugar in the ensilaged mass. The lactic acid has very strong bactericide but weak fungicide properties. In contrast, acetic, byteric and propionic acid have expressed fungicide effects, so the lesser amount of these acids is even desirable in maize silages, as in the other forage species silages, (Table 3).

Table 3. Biochemical changes in alfalfa silages *(Avasi et al., 1999)*

Parameters	Control	Sil-All	Feed Tech
PH	5.12	4.75••	4.82••
Lactic acid, %	1.86	2.76••	2.21••
Acetic acid, %	0.74	0.98•	0.81••
i- butyric acid, %	0.015	0.015	0.0120
n- butyric acid, %	0.035••	0.00	0.00
Propionic acid, %	0.065••	0.015	0.015
NH3-N/∑N, gkg^{-1}	157.0•	150.2	130.7

Sil-All=*Streptococcus faecium, Pediococcus acidilactici, Lactobacillus plantarum*+ enzymes cellulase; Feedtech=*Pediococcus acidilactici*+*Lactobacillus plantarum,* $2,5\times10^8$ cell/g. *p<0,05; **p<0,01

The significance of modern mechanization for the legumes ensilaging

One of the modern methods in the technology of feed ensilaging is the preapiring of haylage in bales, wrapped in foils. Currently in the United States, the most economic way of ensilaging is in the form of silo tubes. This does not require the existence of the specialized silo buildings. The prepared bales and silo tubes can be stored under the eaves, or on the field, to the moment of use. Dried plant material that is baled should contain as little moisture as possible, because it is compacted using the press, and it can be further treated with chemicals or inoculates in order to get the better quality. The great advantage of haylage prepared in bales is that it is easy to handle. Due to this, feed that is ensilaged this way is suitable for market *(Dinic and Djordjevic, 2005)*. Results of research

conducted so far indicate that the equal quality of alfalfa silages is achieved by ensilaging in bales and by ensilaging in the classic silo buildings (*Jatkauskas and Vrotniakiene, 2003,* Table 4).

Table 4. Chemical composition of silages ensiled by different methods, feed intake and animal performance in the experimental period *(Jatkauskas and Vrotniakiene, 2003)*

Parameters	Silage made in trench	Silage made in big bale	SEM	P
pH	4.52	4.65	0.1	NS
Lactic acid	22.1	21.7	0.9	NS
Acetic acid	17.6	14.5	1.2	NS
Butyric acid	0.01	0.00	0.0	NS
NH_3-N, g kg^{-1} N	50.8	45.6	2.4	NS
ME, MJ kg^{-1} DM	8.89	8.94	0.9	NS
NE intake, MJ day^{-1}	88.91	98.82	-	-
Silage intake, kg DM	7.19	7.26	-	-
Initial live weight, kg	356.33 ± 18.64	354.60 ± 19.17	-	-
Total live weight, kg	159.44 ± 14.13	163.50 ± 4.35	-	-
Average live weight gain, kg day^{-1}	1.115 ± 0.09	1.143 ± 0.03	-	-

Conclusion

Modern trends in technology of legumes ensilaging are based on the latest scientific and technical achievements. Different procedures are first tested in the experiments, and then introduced into practice. Numerous researches were conducted in our country in accordance with specific conditions, and the acquired knowledge is translated into concrete recommendations, which are largely applied in our practice. Biomasses of both perennial and annual legumes were studied from the aspect of suitability for ensilaging and of the content of water-soluble carbohydrates and buffer capacity. The effect of increasing the content of dry matter (by wilting) on the quality of fermentation and preservation of nutritional substances was, also, studied. Influence of the share of biomass of legumes that are easy to ensile, the impact of share of annual legumes and grain crops on the fermentation quality and nutritive value of silage was studied. The aim of using modern methods of ensilaging is to get the silage of the highest quality from different raw materials, with the optimal satisfaction of a number of nutrient requests, and to produce the maximum healthy products of animal origin for the human use.

Trendovi u siliranju leguminoza

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Rezime

Savremeni trendovi u tehnologiji siliranja leguminoza zasnivaju se na poznavanju biomasa sa aspekta pogodnosti za siliranje, provenjavanju, dodavanju ugljenohidratnih hraniva, upotrebi bioloških dodataka, i dr. Trenutno se u svetu eksperimentiše sa inokulantima, koji pored homofermentativnih, sadrže i heterofermentativne bakterije mlečne kiseline. Produkti ovakvih inokulanata doprinose povećanju aerobne stabilnosti silaža, pa su navedeni dodaci aktuelni za sve vrste silaža. Osim korišćenja navedenih dodataka, savremena tehnologija siliranja se bazira na maksimalnoj mehanizovanosti celokupnog procesa siliranja, kao i pripremanja silaže u formi roto-bala i silokobasica (najeftiniji vid konzervisanja) kao i stalnih objekata.

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ESTIMATION OF RED CLOVER (*Trifolium pratense* L.) FORAGE QUALITY PARAMETERS DEPENDING ON THE STAGE OF GROWTH

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Abstract: The objective of this study was to investigate the changes that take place in nutrient values of red clover (*Trifolium pratense* L.) *cv* K-27 at different stages of growth in the second and the third cut. The samples from different stages of growth: mid-bud stage, around 60% flowering and full flowering were investigated for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), total digestible nutrients (TDN), net energy-lactation (NEl), net energy-maintenance (NEm) and net energy-gain (NEg) content. Higher content of crude protein was found in the third cut (262.1 g kg^{-1} of DM) than in the second cut (260.8 g kg^{-1} of DM) in the first stage of development. TDN, DDM, DMI, RFV, NEl, NEm and NEg were calculated according to the appropriate equations adapted from common formulas for forages. The stage of plant development has a significant influence on the chemical composition and the relative feed value of red clover. The achieved results show that adequate maturity stage for cutting might be at mid-bloom stage, when crude protein content, NEl, NEm and NEg content are high.

Key words: red clover, forage quality, nutritive value, stage of growth

Introduction

Red clover is a legume well adapted to poorly drained soils and more tolerant to lower soil pH and fertility than alfalfa. The yield potential of red clover is excellent and some red clover varieties can have higher fodder yields than alfalfa. Red clover is considered a short-lived legume, but new modern varieties are productive for three full seasons. Red clover is also of a very good quality as to its nutritive value and ensiling *(Hoffman and Broderick, 2001)*.

The possibility to accurately predict the nutritive value of forage crops is a prerequisite for designing rations and directing forage crops breeding *(Kruse et al.,*

2008). Nutrient deficiencies in low quality roughages affect microbial growth and fermentation in the rumen and result in an overall low animal productivity. Quality forage promotes good health and better performance *(Harris, 1992)*. Therefore, choosing adequate stage of growth of forages with good quality is very important for animal health as well as high milk and meat production.

Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), relative feed value (RFV) and total digestible nutrients (TDN) are important criteria for determining hay quality *(Yolcu et al., 2008)* in animal feeding. Therefore, CP, ADF, NDF, DDM, DMI, RFV, TDN and NEl *(Lithourgidis et al., 2006)* were examined as quality criteria in many studies.

This study was conducted in order to investigate the changes that take place in nutrient values (CP, NDF, ADF, DDM, DMI, RFV, TDN, NEl, NEm and NEg) of red clover, *cv* K-27 at different stages of growth in the second and the third cut.

Materials and Methods

The experiment was carried out in the experimental field of Institute for forage crops in Kruševac in 2008 as a factorial trials, by randomized block system in three replicates. Three stages of growth of red clover (*Trifolium pratense* L.) *cv* K-27 were examined in the second and third cut. The first stage was cut after 22 days of the first cut, at mid-bud stage, another one after 29 days of the first cut, at around 60% flowering, and the third one in full flowering after 36 days of the first cut.

Crude protein (CP) were computed indirectly from the amount of total nitrogen, measured by the Kjeldahl method modified by Bremner, multiplied by factor 6,25. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyses were determined by *Van Soest (1963)*. Digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), total digestible nutrients (TDN), net energy-lactation (NEl), net energy-maintenance (NEm) and net energy-gain (NEg) were calculated according to the equations adapted from common formulas for forages *(Schroeder, 1994)*.

Data were processed by the analysis of variance in a randomized block design. Effects were considered different based on significant ($P< 0.01$) F ratio. The significance of differences between arithmethic means was tested by LSD test.

Results and Discusion

The results of this investigation indicated that the crude protein content of red clover declined with advancing maturity in the second and third cut (Table 1). Crude protein content of plant tissue reflected the relative proportions of each tissue type, and was considered with other findings for red clover *(Hoffman et al., 1993)*.

The NDF and ADF concentrations of forages provide useful information about quality. The results of this study show that contents of cell wall increased in both investigated cuts (Table 1). During maturation NDF content increased from 335.9 to 422.9 g kg^{-1} of DM in the second and from 325.9 to 399.5 g kg^{-1} of DM in the third cut ($P< 0.01$). This results is in agreement with the report of *Kamalak et al. (2005)* who found that NDF and ADF content increased.

Forage quality declines with advancing maturity. Significant differences were determined among forage stages of growth in terms of DDM, DMI and RFV for animal feeding (Table 1). The highest DDM ratio was found at the first stage of plant development (70,1% in the second cut, and 75,7% in the third cut). The lowest DDM ratio was found at the third stage of plant development in both cuts. Maturity at harvest also influences forage consumption by animals. This is because NDF is more difficult to digest than the non-fiber components of forage. The RFV of red clover hay harvested at different maturity stages ranged from 194.3 to 140.9 in the second cut and from 216.0 to 153.7 in the third cut. This values are higher than values reported by *Stallings (2005)*. The decrease in DDM, DMI and RFV are possibly associated with increased NDF and ADF contents *(Wilson et al., 1991)* and increased lignification and decreased leaf:stem ratio *(Hides et al., 1983)* as the plant matures.

Table1. Forage quality of red clover (*Trifolium pratense* L.) *cv* K-27 at different maturity stages in the second and third cut

	CP g kg^{-1}	NDF g kg^{-1}	ADF g kg^{-1}	DDM %	DMI %	RFV %
			II cut			
I stage	260.8 a	335.9 b	240.7 c	70.1 a	3.5 a	194.3 a
II stage	219.6 b	364.4 b	286.1 b	66.6 b	3.3 a	170.1 a
IIIstage	173.8 c	422.9 a	318.8 a	64.1 c	2.8 b	140.9 b
			III cut			
I stage	262.1 a	325.9 c	169.9 c	75.7 a	3.7 a	216.0 a
II stage	180.2 b	382.5 b	239.9 b	70.2 b	3.1 b	170.8 b
IIIstage	159.3 c	399.5 a	294.2 a	65.9 c	3.0 c	153.7 c

Different letters within a column denote significantly different means ($P< 0.01$)

TDN refers to the nutrients that can be utilized and available for livestock *(Lithourgidis et al., 2006)*. Differences (P< 0.01) in terms of TDN contents were determined among stages of growth in both cuts. Total digestible nutrients contents of red clover ranged from 68.7 to 59.7 % in the second cut, and from 76,8 to 62.5% in the third cut (Table 2). The highest total digestible nutrients contents were determined at the first stage of growth in the second and the third cut. It is important to provide cattle with adequate amounts of digestible energy for optimal animal performance. While many factors affect forage digestibility and ultimately TDN, the primary factor producers can control is forage maturity.

The energy concentration, expressed for dairy cows in MJ NEl kg^{-1} DM is very important. The highest possible level of energy concentration is a prerequisite to feed highly performing cows successfully. But possibilities to increase cows energy intake by increasing the energy concentration are limited. The highest NEl content in the second cut was recorded at the first stage of development (6.54 MJ kg^{-1} of DM), and the lowest was in the third stage of growth (5.62 MJ kg^{-1} of DM). In the third cut NEl content ranged from 7,38 to 5,92 MJ kg^{-1} of DM. The enhancement of NEl value is very important, as *Hrabe et al. (2003)* reported that an increase of NEl of clover-grass silage by 0.5 MJ kg^{-1} of DM reduces the need for complementary feed by 2.5 kg per day.

The energy value of feed in our trial expressed by NEm values varied from 7.12 to 6.03 MJ kg^{-1} of DM in the second and from 8.10 to 6.37 MJ kg^{-1} of DM in the third cut. The differences between stages of growth were significant (P< 0.01).

Significant differences between stages of growth were recorded for NEg in this study (P< 0.01). The highest content of NEg was at the first stage of plant development (4.11 MJ kg^{-1} of DM in the second cut, and 5.09 MJ kg^{-1} of DM in the third cut). Higher content of NEg was determined in the third cut than in the second cut (Table 2).

Table 2. Nutritive value of red clover (*Trifolium pratense* L.) *cv* K-27 at different maturity stages in the second and third cut

	TDN %	NEl MJ kg^{-1}	NEm MJ kg^{-1}	NEg MJ kg^{-1}
		II cut		
I stage	68.7 a	6.54 a	7.12 a	4.11 a
II stage	63.5 b	6.01 b	6.48 b	3.47 b
IIIstage	59.7 c	5.62 c	6.03 c	3.02 c
		III cut		
I stage	76.8 a	7.38 a	8.10 a	5.09 a
II stage	68.7 b	6.55 b	7.13 b	4.12 b
IIIstage	62.5 c	5.91 c	6.37 c	3.36 c

Different letters within a column denote significantly different means (P< 0.01)

Energy is often referred to digestible energy, net energy for maintenance (NEm), net energy for gain (NEg), net energy for lactation (NEl), and total digestible nutrients (TDN). When digestible energy becomes limiting in beef cattle diets, intake and animal performance can suffer. Signs of energy deficiency include lowered appetite, weight loss, poor growth, depressed reproductive performance, and reduced milk production. Providing adequate digestible energy in beef cattle diets is important for animal health and productivity as well as ranch profitability.

Conclusion

The choice of harvesting moment is important insuring the forage crops quality and quantity. Relative feed value might be a relevant indicator supporting the decisions of cutting management, especially when red clover is a component of a legume-grass mixture. This experiment showed a decresing RFV of the red clover, *cv* K-27 with plant development. In the second cut the grade of red clover hay at the first and the second stage of development was prime, but at the third maturity stage it was premium - 1. In the third cut, at all three maturity stages the grade of red clover hay was premium. Significant differences were determined in terms of CP, NDF, ADF, DDM, DMI, TDN, NEl, NEm and NEg. From our results we may conclude that adequate maturity stage for cutting might be mid-bloom stage, when crude protein content, NEl, NEm and NEg content are high.

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Procena parametara kvaliteta crvene deteline (*Trifolium pratense* L.) u zavisnosti od faze razvića

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Rezime

Cilj ovog rada je bio ispitivanje promena hranljive vrednosti crvene deteline (*Trifolium pratense* L.) sorte K-27 u različitim fazama razvića u drugom i trećem otkosu. Uzorci iz različitih faza razvića: sredina butonizacije, oko 60% cvetanja i puno cvetanje su ispitivani na sadržaj sirovih proteina, neutralne

deterdžent celuloze, kisele deterdžent celuloze, svarljive suve materije, konzumiranja suve materije, relativne hranljive vrednosti, ukupnih svarljivih hranljivih materija, neto energije laktacije, neto energije za održavanje i neto energije prirasta. Veći sadržaj sirovih proteina (262,1 g kg^{-1} SM) je ustanovljen u trećem, nego u drugom otkosu (od 260,8 kg^{-1} SM) u prvoj fazi razvića. Sadržaj ukupnih svarljivih hranljivih materija, svarljivosti suve materije, konzumiranja suve materije, relativne hranljive vrednosti, neto energije laktacije, neto energije za održavanje i neto energije prirasta su izračunate prema odgovarajućim formulama za kabasta hraniva. Ustanovljeno je da faza razvića ima značajan uticaj na hemijski sastav i relativnu hranljivu vrednost crvene deteline. Dobijeni rezultati pokazuju da adekvatna faza razvića za košenje crvene deteline može biti sredina cvetanja kada je sadržaj sirovih proteina, NEl, NEm i NEg visok.

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EFFECT OF CROP DENSITY ON YIELD AND QUALITY OF ALFALFA FORAGE FROM COMBINED USE (FORAGE-SEED)

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Abstract: In Serbia, alfalfa is the most important perennial forage legume that is grown at 180-200 thousand hectares. Except for the classic production of forage, alfalfa is very important for seed production. In our conditions Alfalfa seed are produced from the second cut of alfalfa. Higher seed yields are achieved in larger distances between rows. In this system of production first and third cut are used for fodder. This trial tests three different densities and four varieties in order to determine the impact on yield and quality of forage dry matter. In the year of establishment, cultivars from the treatment A_1 achieved higher yields of dry matter for 2.3 t ha^{-1} in relation to the treatment A_2. In the second year, yield was higher by 1.4 t ha^{-1}, and in the third higher for 3.1 t ha^{-1}. Treatment A_1 compared to A_3 treatment had a higher yield of 3.5 t ha^{-1} (52%). In the second year treatment A_1 achieved higher yields of forage then treatment A_3 for 3.8 t ha^{-1} (44%), and in the third year for 4.6 t ha^{-1} (56%). Cultivars were also significantly affected the yield of forage dry matter at all densities. Influence of density and variety to the chemical composition of forage and the content of macro elements in the forage had no significant effect.

Key words: alfalfa, crop density, forage, combined use

Introduction

Alfalfa, as the most important perennial forage legume, is cultivated in Serbia at about 200 thousand hectares. In seed production of alfalfa, first and third growth usually used to forage production and the second for seed. Alfalfa seed production requires low plant densities in the year of establishing and a small amount of seed for sowing *(Askarian et al., 1995; Beković et al., 2005 and 2008; Zhang et al., 2008)*. However, getting more seeds based on low densities impact on

reducing forage yield of alfalfa in the first and third growth *(Vučković, 1992; Beković, 2005)*.

It seems that it is very important to find the optimal crop density for the best compromise between yield of seed obtained from second growth and yield of forage obtained from the first and third growth. Domestic cultivars are the most represented in Serbia, for the both productions – forage and seed. Foreign varieties represented in the minimal surfaces.

Previous studies in the world and in our country showed that proper selection of varieties and optimum use of interaction cultivar and external environment can significantly affect the forage yield *(Annicchiarico and Pecetto, 2010)*. In our conditions, however, it often happens that (due to the strong drought in August and September) alfalfa not achieved the third growth and had no forage yield in the fall.

The aim of this study was to find how the combined (forage-seed) production of different varieties of alfalfa influenced on the forage yield obtained from the first and third growth, chemical composition, and the content of macro elements.

Materials and Methods

A trial was conducted in 2002-2004 on soil type smonitza in the agro-ecological conditions of Zajecar (43^0 53' N; 22^0 17' E and 159 m a.s.l.). Randomized block design with four replications used.

Crop density, as the first factor (factor A), had three treatments:

- 18 kg ha^{-1} seed and inter-row spacing of 20 cm (A_1),
- 9 kg ha^{-1} seed and inter-row spacing of 50 cm (A_2),
- 4.5 kg ha^{-1} seed and inter-row spacing of 80 cm (A_3).

Variety was the second factor and we used three domestic cultivars: (Novosadjanka H-11 (B_1); NS-Slavija (B_2); Zaječarska-83 (B_3) and one French variety Europe (B_4).

Sowing was in mid-April 2002. In the year of sowing the seeds usually have low yields, and forage yields were determined from the two cuts. In the first year of use (2003) because of pronounced dry period in summer (Table 1) the third cut was reduced (C_3). In the second year of use (2004) the yield and quality of forage were determined from the first (C_1) and third growth (C_3).

Chemical composition and content of mineral nutrients were done in four replicates and component concentrations were corrected to a 100º C dry matter basis. Energy feeding was also calculated according to the appropriate equations adapted from common formulas for forages. Using standard methods of chemical analysis was determined:

- Crude protein (CP),
- Crude fiber (CF),
- Nitrogen free extract (NFE),

- Phosphorus (P),
- Potassium (K),
- Calcium (Ca),

Statistical analysis of results was performed using the SPSS software *(SPSS, 2000)*.

Table 1. Agroecological conditions in Zajecar

Month	Average month temperatures (°C)			Precipitations (l m^{-2})		
	2002	2003	2004	2002	2003	2004
January	0.4	-0.3	-2.1	15.7	53.1	90.6
February	6.1	-3.3	2.2	1.9	19.4	41.8
March	8.8	4.2	6.6	15.3	7.8	46.6
April	10.3	10.2	11.9	50.3	89.0	46.4
May	18.6	22.9	14.9	45.2	60.5	27.6
June	22.6	22.5	19.5	43.5	43.3	81.3
July	23.8	22.3	21.9	117.3	55.6	49.0
August	20.8	24.3	20.5	118.0	1.3	62.1
September	15.7	15.6	15.9	73.1	67.6	35.6
October	10.6	9.1	12.2	53.6	149.0	45.9
November	6.6	6.5	6.6	30.3	27.2	78.6
December	-2.2	-0.5	1.9	63.3	35.7	34.8
Year average	11.8	11.1	11.0	627.5	609.5	641.2
Period (IV-IX)	18.6	19.6	17.4	447.4	317.3	302.0

Results and Discussion

Eastern Serbia is characterized by a pronounced dry periods in summer (Table 1), which is generally good for seed production, but in the same time these conditions affect on the reducing forage growth. Or even the lack of growth as it was in 2003.

Table 2. Statistical probabilities of *F* test for years, main effects, and their interactions on yield of dry forage, and content of CP, CF, NFE, P, K, Ca in forage

Source	df	Forage dry matter (t ha^{-1})	Content in dry matter (g kg^{-1} DM)					
			CP	CF	NFE	P	K	Ca
Year (Y)	2	**	NS	NS	NS	NS	NS	NS
Density (G)	2	**	NS	NS	NS	NS	NS	NS
Cultivar (C)	3	**	NS	NS	NS	NS	NS	NS
Yx G	4	**	NS	NS	NS	NS	NS	NS
Yx C	6	**	NS	NS	NS	NS	NS	NS
Gx C	6	**	NS	NS	NS	NS	NS	NS
Yx GxC	12	**	NS	NS	NS	NS	NS	NS

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. †NS, not significant.

Crop density, cultivar and year, as well as their interaction had a significant effect ($p \leq 0.01$) on forage yield (Table 2). The highest density achieved the highest yield of forage in all years (Table 3).

Treatment with the highest quantity of seeds in the sowing and the lowest inter-row spacing, compared to the average density, achieved in the first year (average of all varieties) better yield of dry forage for 2.3 t ha^{-1}; in the second year for 1.4 t ha^{-1}; and for 3.1 t ha^{-1} in third year. Also the highest density (treatment A_1) compared to the A_3 treatment had a higher yield for 3.5 t ha^{-1} (or by 52%); over the second year for 3.8 t ha^{-1} (44%), and for 4.6 t ha^{-1} in the third year (or 56%).

Table 2. Dry matter yield (t ha^{-1}) from combined production

Year	2002			2003			2004		
Density / Cultivar	A_1 C_1+C_2	A_2 C_1+C_2	A_3 C_1+C_2	A_1 C_1	A_2 C_1	A_3 C_1	A_1 $C_{1+}C_3$	A_2 $C_{1+}C_3$	A_3 $C_{1+}C_3$
A1	7.9ab	5.4b	3.9b	6.7ab	5.4b	2.9b	10.3a	7.3a	5.6b
A2	8.1a	5.8a	4.2a	6.9a	5.5a	3.1a	10.9a	7.5a	6.3a
A3	8.4a	5.9a	4.4a	7.0a	5.4a	3.2a	10.7a	7.6a	6.5a
A4	7.1b	5.1c	3.7b	6.6b	5.1b	2.8b	9.5b	6.8b	4.6c
Average	7.9	5.6	4.1	6.8	5.4	3.0	10.4	7.3	5.8

LSD significant level for column 5%.

In all years and all densities Zaječarska-83 and NS-Slavija showed superiority for forage yield. There is no significant difference between them ($p \leq 0.05$). In relation to the cultivar Europe, cultivars NS-Slavija and Zaječarska-83 had a significantly higher yield of forage during all three years with all densities. In compare to NS H-11, cultivars NS-Slavija and Zaječarska-83 did not show significant difference ($p \leq 0.05$) only in 2004 at the highest and high densities.

Several researchers pointed out that there are significant differences between domestic and foreign cultivars of alfalfa dry matter yield and forage production, and given the priority to domestic varieties, which are generally associated with better adaptability of local varieties. So *Svirskis (2003)* examined the yield of 12 varieties of alfalfa with different backgrounds (three from the Czech Republic, six from France, one from the Slovak Republic, two from Canada, and two from Lithuania). The highest dry matter yield was achieved with local varieties Birute and Žydrune (20.6 and 20.9 tha^{-1}). With the lowest yield was Canadian Alfagraze.

Table 3. Effect of density and cultivar on chemical composition dry matter forage yield (2002-2004)

Trait	CP (g kg^{-1} DM)			CF (g kg^{-1} DM)			NFE (g kg^{-1} DM)		
Density / Cultivar	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
B_1	199	198	198	258	269	269	421	410	409
B_2	200	200	199	259	263	264	417	413	411
B_3	199	198	197	259	262	270	417	413	408
B_4	193	193	190	269	269	273	413	414	413
Average	198	197	196	261	266	269	417	413	410
CV (%)	1.62	1.51	2.08	1.99	1.42	1.39	0.78	0.42	0.54

Examined factors yield, density and variety as well as their interaction did not significantly ($p \leq 0.05$) the content of crude protein, crude fiber and nitrogen free extracts (Table 2, and 3).

However, the forage of the treatment A_1 compared to forage from treatment A_3 (average of all varieties), over the three years period had a higher content of CP for 2 g kg^{-1} DM, lower content of CF for 8 g kg^{-1} and higher content of NFE for the 7 g kg^{-1} DM.

Similar results of density impact on CP content showed *Haby et al. (1999)*, in which the alfalfa grown in the inter-row distance of 23 cm had a crude protein content of 211.0 g kg^{-1} DM, respectively, and at 92 cm inter-row distance 203 g kg^{-1} SM. According to *Đukić (2002)*, effect of cultivar on CP content was higher (Leninskaja - 212 g kg^{-1}, NS Mediana - 188.0 g kg^{-1} DM).

Results about content of the CF and NFE in DM depending on the density and cultivars are similar with the results of *Negovanović et al. (1992)* and *Mišković et al (1975), 435.4-414.1 g kg-1 DM.*

Also, the density and variety impact as well as their interaction did not significantly ($p \leq 0.05$) affect the macro elements content in alfalfa forage (Table 4). However, the crop with high density (A_1) compared to low density (A_3) was found slightly higher concentration of P (for 0.02 g kg-1 DM), the concentration of K (0.6 g kg^{-1} DM) and Ca (0.5 g kg^{-1} DM).

Table 4 Effect of density and cultivar on macro element concentration in dry matter (g kg^{-1} SM) (2002-2004)

Trait	P (g kg^{-1} DM)			K (g kg^{-1} DM)			Ca (g kg^{-1} DM)		
Density / Cultivar	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
B_1	2.41	2.40	2.40	14.8	14.7	14.5	14.8	14.7	14.5
B_2	2.44	2.42	2.41	15.3	14.9	14.6	15.1	14.9	14.6
B_3	2.43	2.43	2.41	15.3	14.5	14.4	15.3	14.7	14.5
B_4	2.40	2.40	2.39	14.9	14.5	14.5	14.9	14.6	14.5
Average	2.42	2.41	2.40	15.1	14.7	14.5	15.0	14.7	14.5
CV (%)	0.75	0.62	0.40	1.74	1.31	0.56	1.48	0.85	0.34

Depending on the variety (the average density for all) the content of P differed for 0.03 g kg^{-1} DM, content of K for 0.5 g kg^{-1} DM and Ca content for 0.1 g kg^{-1} DM.

The results about the crop density impact on the concentration of macro elements are similar to *Mišković (1975)*, (K = 15.0 g kg^{-1} DM, Ca = 17.3 g kg^{-1} DM). While *Djukić (2002)* reported significant effects of genotype on microelement concentration in alfalfa dry matter forage.

Conclusion

Alfalfa forage production from the combined use (forage-seed) and a classic crop (12 -24 cm inter row density) provides less density and inevitably leads to a significant reduction in dry matter forage yield. Using of interaction of cultivar x environmental conditions and proper choice of cultivar could have a significant impact on the yield of dry matter forage crops at all densities.

Year, crop density, cultivar and their interactions did not show significant effect ($p \leq 0.05$) on the chemical composition and concentration of macro elements in the forage. However, althout they were not statistically significant, varieties NS-Slavija and Zaječarska-83 with higher density, showed better quality of forage.

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Uticaj gustine useva na prinos i kvalitet krme lucerke iz kombinovanog korišćenja (krma-seme)

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Rezime

Lucerka, kao najznačajnija krmna leguminoza se u Srbiji gaji na 180-200 hiljada hektara. Osim klasične proizvodnje za krmu, u širokoj proizvodnji je uobičajeno da se lucerka koristi kombinovano za seme i krmu, pri čemu se prvi i treći otkos koriste za krmu, a drugi otkos za seme. Semenska proizvodnja zahteva manju gustinu biljaka u godini zasnivanja i malu količinu semena za setvu, međutim, manja gustina useva utiče na smanjenje prinosa krme u prvom i trećem otkosu.

U ovom radu su ispitivane tri različite gustine i četiri sorte lucerke Novosađanka H-11 (B_1); NS-Slavija (B_2); Zaječarska-83 (B_3) i francuska sorta Europe (B_4), da bi se ispitala interakcija između sorti, gustina i ekoloških uslova na zadovoljavajući prinos i kvalitet kako krme, tako i semena.

Korišćenjem interakcije sorta x spoljna sredina može se značajno uticati na prinos suve materije krme pri svim gustinama useva. U poređenju sa sortom Europe, sorte NS-Slavija i Zaječarska-83 su imale signifikantno veći prinos krme tokom sve tri godine i pri svim ispitivanim gustinama, što se može objasniti boljom adaptabilnošću domaćih sorti.

Godine, gustina useva, sorta, kao i njihove interakcije nisu pokazale značajan ($p \leq 0.05$) uticaj na hemijski sastav i koncentraciju makroelemenata u krmi. Međutim, iako nije statistički značajno ipak je na usevu iz gustorednog useva (tretman A) kod sorti NS Slavija i Zaječarska - 83 utvrđen nešto bolji kvalitet krme.

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APPEARANCE AND FREQUENCE OF FUNGI ON ALFALFA SEED IN SERBIA

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Abstract: Alfalfa is one of the most significant forage crops in Serbia, and is known colloquially as "the queen of forage crops" due its significant role in livestock nutrition. Production of high quality forage and seed is of high importance for animal husbandry in Serbia. Fungi infections decrease seed quality which has influence on yield and quality of forage and on period of alfalfa utilization. Samples of alfalfa seed from Middle Banat, Danube region, Niš and Šumadija area were examined. Samples were taken from two alfalfa cultivars K-22 and K-28. Based on the examinations of appearance of fungi on the alfalfa seed, following fungi were determined: *Fusarium* spp., *Alternaria* spp., *Stemphylium* spp., *Epiccocum* spp., *Nigrospora* spp., *Rhizopus* spp., *Mucor* spp., *Aspergillus* spp. and *Penicillium* spp. Primary species were from genera *Fusarium* and *Alternaria*, and other species were represented in different percentages.

Key words: seed, fungi, alfalfa

Introduction

A large number of pathogens that cause diseases of root and above-ground parts of alfalfa are usually transmitted by seed. Apart from pathogenic microorganisms, often are saprophytic microorganisms on alfalfa seed. For these reasons, especially when it comes to perennials, such as alfalfa, it is important to use healthy seed for sowing. The most common pathogenic and saprophytic microorganisms on alfalfa seed are fungi and less common are bacteria and viruses. Some pathogenic bacteria and fungi maintain vitality in the soil and the plant remains for years, and are a source of infection for the planted seed and alfalfa seedlings.

In Serbia, over ten different types of fungi were isolated from alfalfa and red clover seed, of which most of the genera: *Alternaria* and *Fusarium (Krnjaja et al., 2003, 2004)*.

Due to its importance in the implementation of healthy seeds during the establishing of perennial forage legumes, it is important to study the occurrence and intensity of fungal diseases on alfalfa seeds.

Materials and Methods

Research was conducted in the Laboratory for Microbiology of the Institute for Forage Crops in Kruševac. As a starting material two varieties of alfalfa were used: K-22 and K-28. The samples originated from the harvest in 2009. Sampling was conducted during period of February-March 2010. Plant material was derived from areas of Serbia (Central Banat district, the Danube district, Niš district, Šumadija district).

Several samples were taken from each district and 100 seeds were used per sample in 10 Petri dishes, 10 seeds per Petri dish.

In the method A, the seeds were sterilized by the method of *Graham et al. (1976)*. Seeds were surface-sterilized by immersing in 0.26% sodium hypochlorite for five minutes and then dried. Ten seeds were placed in each Petri dish with on corn meal agar medium (CMA). Dishes were kept at room temperature for 10 days.

In the method B, the seeds were surface sterilized by the method of *Chi et al. (1964)*. Seeds were surface disinfected in 7% sodium hypochlorite (NaOCl) for 5 minutes, rinsed in sterile water and dried at room temperature. Then the seeds were put on potato dextrose medium (PDA).

After ten days, the colonies were developed around the seed, and each colony was separately examined under the microscope for future identification and some of the cultures were transferred to two media: potato-dextrose medium (PDA) and the medium prepared from water and agar (WA).

Determination of *Fusarium* species was conducted based on macroscopic and microscopic traits on the PDA and water-agar medium (WA) according to the methods of *Nelson et al. (1983)* and *Burgess et al. (1994)*, while other genera were determined by the method of *Watanabe et al. (1994)*.

Results

Microscopic study of colonies of fungi, isolated from alfalfa seed, showed varying degrees of infection, at different times and way of seed sterilization (Table 1). There was a significant difference in health status of seed using methods A and B.

Table 1. The intensity of alfalfa seed infection

Cultivar	Intentsity of seed infection (%)			
	Methods A		Methods B	
	Fungus type	%	Fungus type	%
K-22	*Alternaria* spp.	25.2	*Alternaria* spp.	10.2
	Aspergillus spp.	2.8	*Aspergillus* spp.	2.0
	Cladosporium spp.	3.8	*Cladosporium* spp.	-
	Epiccocum spp.	1.8	*Epiccocum* spp.	-
	Fusarium spp.	26.6	*Fusarium* spp.	12.1
	Mucor spp.	4.6	*Mucor* spp.	3.0
	Nigrospora spp.	3.5	*Nigrospora* spp.	-
	Penicillium spp.	3.6	*Penicilliium* spp.	1.0
	Rhizoctonia spp.	0.5	*Rhizoctonia* spp.	-
	Rhizopus spp.	4.1	*Rhizopus* spp.	3.0
	Sclerotinia spp.	0.9	*Sclerotinia* spp.	-
	Stemphylium spp.	14.7	*Stemphylium* spp.	-
	Trichoderma spp.	1.8	*Trichoderma* spp.	-
K-28	*Alternaria* spp.	25.0	*Alternaria* spp.	12.0
	Aspergillus spp.	9.0	*Aspergillus* spp.	-
	Cladosporium spp.	2.9	*Cladosporium* spp.	-
	Epiccocum spp.	0.8	*Epiccocum* spp.	-
	Fusarium spp.	28.8	*Fusarium* spp.	15.0
	Mucor spp.	7.5	*Mucor* spp.	1.0
	Nigrospora spp.	4.8	*Nigrospora* spp.	-
	Penicillium spp.	2.9	*Penicilliium* spp.	1.1
	Rhizoctonia spp.	-	*Rhizoctonia* spp.	-
	Rhizopus spp.	5.5	*Rhizopus* spp.	0.5
	Sclerotinia spp.	2.7	*Sclerotinia* spp.	-
	Stemphylium spp.	7.3	*Stemphylium* spp.	-
	Trichoderma spp.	1.8	*Trichoderma* spp.	-

Based on the obtained results we can conclude that different time and way of sterilization affect the degree of seed infection.

The method A involves a shorter time of sterilization, as evidenced by the number of fungi, where total of thirteen genera were isolated. There is a difference in the percentage of representation of fungi in K-22 and K-28. The most numerous fungi of the genus *Fusarium* (26.6%) in the variety K-22, while in K-28 was 28.8% and *Alternaria* (25%) for the variety K-22 while for K-28 was 25.2%. *Stemphylium spp*. with 14.7% represented in the variety K-22 and in K-28 was represented with 7.3%. Genus *Rhizoctonia* was not isolated in the variety K-28 while the K-22 was determined for 0.5%. Presence of other fungi genera varied depending on the cultivars, the origin of the sample and the seed storage.

In method B, it can be seen that the six fungi genera isolated in both cultivars (K-22 and K-28) were: *Fusarium* (12.1 and 15.0%), *Alternaria* (10.2 and 12.0%), *Rhizopus* (0.5 and 3%), *Mucor* (1 and 3%), *Penicilliium* (1 and 1.1%).

Different was genus *Aspergillus* isolated in the variety K-22 (2%). The difference was significant in health status of the seed between the methods.

Discussion

Many pathogens of alfalfa (*Medicago sativa* L.) that cause diseases of root and above-ground parts are usually transmitted by seed. Symptoms of the disease to manifest as rot, reduced germination, and wet rot before and after germination, seedlings decay, root and hypocotyl rot (*Hancock,* 1983). Infected seed does not germinate, or even if they germinate, seedlings are dying or give underdeveloped grown plants. The root or the stem initially has watery appearance, and later seedlings die *(Krnjaja et al., 2005)*.

Fusarium spp is, for the most part, weak pathogen and cause little damage to vigorously growing plants. They do, however, penetrate and colonize healthy plants, whether they are wounded or not, and it appears that most alfalfa plants are infected with these fungi at very early age. The amount of damage resulting from the infection depends upon the physiologic condition of the host after infection. Probably any factor that weakness the host predisposes it to be damaged by these fungi *(Krnjaja et al., 2003)*.

According to literature references *Fusarium* species could be considered as potential inducers of diseases in alfalfa. Mentioned species have proved their pathogenicity regarding the root of alfalfa and other hosts when inoculated at the tip of root. Isolates of *Fusarium* spp. constitute, majority, of the fungi isolated from alfalfa roots (up to 90% of all isolates), and isolates *Alternaria* spp., *Penicillium* spp., *Mucor* spp., *Rhizopus* spp., *Trichoderma* spp., *Aspergillus* spp., and the other species, followed in decreasing order of prevalence *(Krnjaja et al., 2003)*.

Alfalfa seedlings are highly susceptible to *Sclerotinia*. Late summer or early fall seedlings are most vulnerable because plants are still in the seedling stage at the time of spore release, whereas spring seedlings will have passed the age of maximum susceptibility *(Stuteville and Erwin, 1990)*.

Stemphylium leaf spot, a common foliar disease of alfalfa, may cause significant defoliation during warm wet periods in summer and fall. Foliar infection by *Stemphylium* reduces root growth and hastens death of alfalfa in the glasshouse, and some evidence indicates that similar effects occur in the field. The fungus *Stemphylium botryousum* is carried on seed *(Stuteville and Erwin, 1990)*.

Obtained results indicate the significance of rinsing of seed (method B) to eliminate the presence of fungi on seed. This measure contributes to improvement of health condition of alfalfa seed. In practice, however, there is no example confirming the actual application of this or similar methods of preventive protection of seed from infection. One of the reasons is insufficient knowledge of

the role of seed as carrier of some pathogen fungi that are important from the economical aspect, including species of *Fusarium* genus.

Conclusion

The species of genera *Fusarium* and *Alternaria* were present in all samples. The presence of other genera depends on sterilization length and origin of the sample.

Considering the significant differences in health state of seed by application of method A and method B, further investigations should be directed towards the development of methods which would show real health state of seed, that is, natural contamination of seed. In this case it is the use of method B.

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Pojava i učestalost gljiva na semenu lucerke u Srbiji

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Rezime

Lucerka je veoma značajna krmna biljka u našoj zemlji, mnogi je nazivaju i "kraljicom krmnih biljaka" jer zauzima značajno mesto u ishrani stoke. Proizvodnja i dobijanje semena lucerke visokog kvaliteta je od primarnog značaja za stočarsku proizvodnju u Srbiji. Zaraze gljivama smanjuju kvalitet semena, a sve to utiče na prinose i kvalitet krme kao i na period iskorišćavanja lucerišta. Ispitivan je veći broj uzoraka semena lucerke poreklom iz: Srednje-Banatskog, Podunavskog, Niškog i Šumadijskog okruga. Uzorci su uzimani sa dve sorte lucerke K-22 i K-28.

Na osnovu proučavanja pojava gljiva na semenu lucerke utvrđeno je da se javljaju sledeće vrste: *Fusarium* spp., *Alternaria* spp., *Stemphylium* spp., *Epiccocum* spp., *Nigrospora* spp., *Rhizopus* spp., *Mucor* spp., *Aspergillus* spp. i *Penicillium* spp. Primarne su bile vrste iz rodova *Fusarium* i *Alternaria,* ostale vrste su bile zastupljene u različitim procentima.

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YIELD AND QUALITY OF WINTER COMMON VETCH (*Vicia sativa* L.) HAYLAGE DEPENDING ON SOWING METHOD

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Abstract: In order to determine an optimum ratio of vetch and wheat (*Triticum aestivum* L.) components in their mixture, there has been carried out a four-year trial (autumn 2005 – spring 2009) aimed at the yield and quality of winter vetch haylage. The sowing rate of winter vetch was 120 kg ha^{-1}, while the sowing rate of winter wheat was 0, 15, 20, 25 and 30 kg ha^{-1}. An increased proportion of the cereal in its mixture with vetch significantly decreases the stand lodging, have a positive influence on forage yield, but haylage quality is of a poorer quality. Quality characteristics such as crude protein and lignin content, total digestible nutrients, dry matter intake and relative feed value were highest in monoculture common vetch followed by mixture with the lowest rate of wheat. Neutral detergent fiber content was positively affected by intercropping. There were no significant differences among treatments for acid detergent fiber content, digestible dry matter, and net energy for lactation. The most favorable balance between the haylage yield and quality, as well as the highest CP yield (1482 kg ha^{-1}), was achieved by the mixture of 120 kg ha^{-1} of the vetch seed and 15 kg ha^{-1} of wheat.

Key words: crude proteins, forage, haylage, intercropping, vetch

Introduction

Winter form of common vetch (*Vicia sativa* L.) is an annual legume crop rich in protein that is traditionally used in the regions of South East Europe as high quality roughage, that is, green forage or hay. Recently it is increasingly used in the form of haylage, due to numerous advantages this way of conservation offers *(Seven and Cerci, 2006)*. Also, the enhanced quality of the conserved forage allowed a greater milk yield and a reduction in the winter feeding costs. Carefully

managing the haylage during storage prevented the risk of clostridial or other bacterial contamination in the milk and produced cheeses *(Borreani et al., 2007)*. However, vetch has a vine growing habit and if sown as monocrop heavily lodges. As a result, forage yield and quality start to decrease due to the decomposition of herbage *(Gulcan et al., 1988; Aydin and Tosun, 1991; Karagić et al., 2008; Mihailović et al., 2007, 2008)*. Because of all this, it is sown with winter-sown small grains, such as oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and triticale (x *Triticosecale* Wittmack).

In mixtures, companion cereals provide structural support for common vetch growth, improve light interception, and facilitate mechanical harvest, whereas common vetch in mixtures improves the quality of forage *(Roberts et al., 1989; Caballero et al., 1995; Lithourgidis et al., 2006)*. So far, there has been no consent opinion on what cereal is the best companion crop for vetch *(Caballero and Goicoechea, 1986; Thompson et al., 1992)*. *Roberts et al. (1989)* reported that the most suitable cereal for mixtures with common vetch is wheat. Due to a problematic response to low temperatures in oats, in Serbia it is wheat that is most often used as a companion crop for winter vetch.

Although numerous studies have examined the effects of varying seeding ratios *(Aydogdu and Acikgoz, 1995; Karadag and Buyukburc, 2003; Kokten et al., 2009; Lithourgidis et al., 2006; Tukel et al.1997; Tuna and Orak, 2007)*, the optimum seeding rates for those combinations are not well-defined. The conclusions these authors have drawn are highly influenced by the climatic conditions their testing had been carried out in. Common vetch produces low yields particularly in areas with low rainfall *(Hadjichristodoulou, 1978)* and seriously hinders harvest because of lodging in areas with high rainfall *(Robinson, 1969; Caballero et al., 1995; Karagić et al., 2008)*.

For the prevailing agro-ecological conditions of Serbia, *Mišković (1986),* recommends that the sowing rates are 150 kg ha^{-1} for common vetch and 30-35 kg ha^{-1} for cereals. However, it has been demonstrated that these sowing rates are too high for the contemporary agriculture *(Karagić et al., 2008)*. By this reason, the objective of this study was to compare the haylage yield and quality from mixed stands of vetch and wheat obtained when several seeding rates of wheat were combined with constant seeding ratio of vetch.

Materials and Methods

A field experiment was conducted during four growing seasons (2005/06, 2006/07, 2007/08, 2008/09) at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, in northern Serbia (45°20'N, 19°51'E). The area has a continental semiarid to semihumid climate, a mean annual air temperature of 11.0°C, an annual sum of precipitation of 617 mm, with an uneven distribution of precipitation. The experiment was established in a loamy soil with pH 7.2, and

organic matter content 2.82 %, N-NO_3 17.2 ppm, P_2O_5 20.7 ppm and K_2O 29.1 ppm (0 to 30 cm depth). Nitrogen and P_2O_5 at 15 and 78 kg ha^{-1}, respectively, were incorporated as monoammonium phosphate (10–52–0) into the soil before ploughing. The previous crop was oilseed rape, with harvest residues chopped and incorporated into the soil.

The experimental design was a randomized complete block with five treatments (vetch monoculture and four mixtures of common vetch with wheat) replicated four times. The sowing rate of vetch in all treatments was 120 kg ha^{-1}, while the sowing rates of wheat were 0 kg ha^{-1} (SRW_0), 15 kg ha^{-1} (SRW_{15}), 20 kg ha^{-1} (SRW_{20}), 25 kg ha^{-1} (SRW_{25}) and 30 kg ha^{-1} (SRW_{30}). The plot size was 60 m^2 (5 x 12 m) and the treatments were separated by a 2 m buffer zone.

The seeds of vetch and wheat were mixed before the sowing that was done in the first decade of October in all four years, with 12.5 cm between rows. Mean seed weights were 50.2 g and 39.8 g per 1000 seeds for common vetch and wheat, respectively. There were applied usual modern agronomic practices of a vetch production. The cutting and chopping of the forage were done by hand with a scythe in the stage of first pods forming and on the area of 10 m^2 in the middle of each plot. The samples of 1 kg biomass from each plot were dried at 65^oC for 72 h in order to determine the dry matter content.

The cut and dried biomass was placed in a silotrench without inoculants. After 60 days of fermenting, the haylage samples of 400 g each were taken, with an analysis of the basic quality parameters. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25 *(AOAC, 1980)*. Neutral and acid detergent fiber (NDF and ADF) and acid detergent lignin (ADL) were determined using the procedure by Goering and van Soest (1970). Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_L) were estimated according to the following equations:

$$TDN = 73.5 + 0.62(\%CP) - 0.71(\%ADF)$$
$$DDM\ (\%) = 88.9 - (\%ADF \times 0.779)$$
$$DMI\ (\%DM) = 120 : \%NDF$$
$$RFV = (\%DDM \times \%DMI) : 1.29$$
$$NE_L = (1.044\text{-}(0.0119 \times \%ADF)) \times 2.205$$

The results were processed by the analysis of variance (ANOVA). The treatment mean differences were separated by the least significant difference (LSD) test at the 0.05 probability level. For the entire statistical analysis the MSTAT-C software was used *(MSTAT-C, 1988)*. Because the analyses of variance for haylage yield and quality indicated no treatment x experimental time interaction, the values are reported as means of the four growing seasons.

Results and Discussion

The increase of the cereal in its mixture with vetch significantly increased the yields of both haylage and DM (Table 1), what agrees with the results by *Roberts et al. (1989)*. The highest vetch haylage yield (18938 kg ha^{-1}) was achieved in the treatment with the highest wheat sowing rate. By decreasing the wheat sowing rate, the haylage yield was also decreasing, that is, from 9.4% in SRW_{25} to 35.4% in the vetch monocrop. There were no significant differences in the vetch haylage yield between the treatments SRW_{15} and SRW_{20}. An identical trend, to that of haylage yield was observed for the dry matter yield (Table 1). The influence of the wheat sowing rate on the vetch proportion in the total yield was significant for all treatments (Table 1).

Table 1. Haylage yield, dry matter yield and vetch contribution of monoculture and mixtures of common vetch with wheat

Treatment	Haylage yield (kg ha^{-1})	Dry matter yield (kg ha^{-1})	Vetch proportion in the total yield (%)
SRW_0	12240 d*	5430 d	100.00 a
SRW_{15}	14970 c	6641 c	80.66 b
SRW_{20}	15366 c	6816 c	74.27 c
SRW_{25}	17151 b	7718 b	62.91 d
SRW_{30}	18938 a	8400 a	56.12 e
Average	15733	7001	74.79
LSD 0.05	1378	623	3.73

*Values within the columns followed by the same letter do not differ significantly at the 0.05 level of probability according to LSD test

The highest CP content was in the vetch monocrop (251.8 g kg^{-1} DM) and with the lowest wheat sowing rate (223.1 g kg^{-1} DM), with no significant differences between these two treatments (Table 2). In all mixtures, the CP content decreased as cereal proportion increased, what responds to the results of numerous authors *(Roberts et al., 1989; Caballero et al., 1995; Balabanli and Turk, 2006; Lithourgidis et al., 2006)*. The decrease of the CP content varied from 30.8% with SRW_{20} to 59.0% with SRW_{30}. The highest CP yield was achieved with SRW_{15} (1482 kg ha^{-1}). Unlike our results, *Tuna and Orak (2007)* recommend the mixture of 25% vetch and 75% of oat, on the basis of solely DM yield (6.5 t ha^{-1}).

The highest NDF content (564.7 g kg^{-1} DM) was in the treatment with the highest wheat sowing rate (Table 2). The decrease of the wheat sowing rate caused the decrease of NDF content, from 7.7% with SRW_{25} to 25.4% in the vetch monocrop. The lowest NDF concentration was in vetch monocrop and the increase of the wheat sowing rate caused the increase of NDF content, which is in agreement with most other studies *(Caballero et al., 1995; Castro et al., 2000; Assefa and Ledin, 2001)*. Having tested the wheat monocrop silage quality, *Siefers*

and Bolsen (1997) determined a relatively low forage quality as evidenced by high NDF and ADF percentages (higher than 60% NDF and 40% ADF contents).

The average ADF content in the vetch haylage was 359.5 g kg^{-1} DM, with no significant differences between the treatments (Table 2). The actual values for ADF found in this study and the lack of significant differences agree with other studies *(Caballero et al., 1995; Castro et al., 2000; Lithourgidis et al., 2006)*.

The lignin content was highest in the vetch monocrop (98.9 g kg^{-1} DM). The increase of the wheat sowing rate from 20 kg ha^{-1} on caused a significant decrease in the lignin content, from 9.2% to 37.6% (Table 2). The results obtained respond to these from other studies *(Caballero et al., 1995, 2001; Rebole et al., 2004; Lithourgidis et al., 2006)*.

Table 2. Crude protein (CP) content and crude protein yield, neutral (NDF) and acid detergent fiber (ADF) and acid detergent lignin (ADL) in haylage of monoculture and mixtures of common vetch with wheat

Treatment	CP (g kg^{-1} DM)	CP (kg ha^{-1})	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)	ADL (g kg^{-1} DM)
SRW_0	251.8 a*	1367 b	421.2 d	356.3 a	98.9 a
SRW_{15}	223.1 a	1482 a	448.4 cd	362.0 a	93.3 ab
SRW_{20}	174.3 b	1188 c	466.9 c	371.9 a	89.2 b
SRW_{25}	123.4 c	952 d	521.3 b	355.4 a	64.6 c
SRW_{30}	103.2 d	867 e	564.7 a	352.1 a	61.7 c
Average	175.2	1171	484.5	359.5	81.5
LSD 0.05	13.8	69	37.5	25.6	9.3

*Values within columns followed by the same letter do not differ significantly at the 0.05 level of probability according to LSD test

The highest TDN was determined in the vetch monocrop (Table 3). There were no significant differences in the TDN content between the treatments SRW_0 and SRW_{15}. The increase of the wheat sowing rate above 15 kg ha^{-1} caused a significant decrease of the TDN content in haylage, from 9.3% to 14.0%, in comparison with SRW_0. Similar values and trends were reported by others where legumes included in the intercropping system significantly increased the TDN *(Osman and Nersoyan, 1986; Roberts et al., 1989)*. However, *Lithourgidis et al. (2006)* found that triticale and oat monocultures had higher TDN than monoculture common vetch, and TDN decreased as the common vetch seeding proportion increased in mixtures. The differences in this research are a consequence of various methods used to determine TDN. The average DDM was 608.9 g kg^{-1} DM, with no significant differences between the treatments (Table 3).

Table 3. Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_L) of the haylage of monoculture and mixtures of common vetch with wheat

Treatment	TDN (g kg^{-1} DM)	DDM (g kg^{-1} DM)	DMI (g kg^{-1} of body weight)	RFV (%)	NE_L (Mcal kg^{-1})
SRW_0	638.1 a*	611.4 a	28.5 a	135.04 a	1.367 a
SRW_{15}	616.3 ab	607.0 a	26.8 ab	126.07 ab	1.352 a
SRW_{20}	579.0 bc	599.3 a	25.7 b	119.37 b	1.326 a
SRW_{25}	559.2 c	612.1 a	23.0 c	109.11 c	1.369 a
SRW_{30}	549.0 c	614.7 a	21.3 c	101.47 c	1.378 a
Average	588.3	608.9	25.0	118.21	1.359
LSD 0.05	43.7	38.6	1.7	9.31	0.114

*Values within columns followed by the same letter do not differ significantly at the 0.05 level of probability according to LSD test

The highest DMI was in the vetch monocrop and the lowest wheat sowing rate, 28.5 and 26.8 g kg^{-1} of BW, respectively. In comparison to the vetch monocrop, the values of the other treatments were significantly lower, from 9.8 to 25.3% (Table 3). The highest RFV was determined in the vetch monocrop and the lowest wheat sowing rate, 135.04 and 126.07%, respectively, which is consistent with results of *Hackman et al. (2008)*. *According to Dunham (1998)* the best use of RFV is for selecting forages to be used in rations which require high nutrient density such as high producing dairy cows. Alfalfa with a RFV less than 140 should not be considered good enough for early lactation cows. However, alfalfa with a RFV of 125 to 140 could be fed to dairy cows in late lactation. Lower RFV alfalfa would be adequate for growing heifers.

The average NE_L was 1.359 Mcal kg^{-1}, with no significant differences between the treatments (Table 3). The different content of common vetch did not affect DDM and NE_L as there were no significant differences among treatments, which is in agreement with results of *Lithourgidis et al. (2006)*.

Conclusion

For forage crops it is important to produce greater forage yields per hectare, higher nutritional quality (percentage composition of selected nutrients) or combined nutrient yields. On the basis of the results obtained by this research, it can be concluded that the most favorable balance between haylage yield and quality, as well as the highest CP yield (1,482 kg ha^{-1}), was achieved by sowing the mixture of 120 kg ha^{-1} of vetch and 15 kg ha^{-1} of wheat, thus recommending this combination to the farmers for the agro-ecological conditions of South East Europe.

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Prinos i kvalitet senaže ozime grahorice (*Vicia sativa* L.) u zavisnosti od načina setve

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Rezime

Ozima grahorica (*Vicia sativa* L.) vrlo je osetljiva na poleganje i zbog toga se seje u smeši sa strnim žitima koja služe kao potporni usev. Povećanjem učešća strnine u smeši sa grahoricom značajno se smanjuje poleganje useva, pozitivno se utiče na prinos krme ali je proizvedena senaža slabijeg kvaliteta. U cilju utvrđivanja optimalnog odnosa strnine i grahorice u travno-leguminoznoj smeši izvršena su četvorogodišnja (2006-2009.) ispitivanja prinosa i kvaliteta senaže ozime grahorice. Setvena norma grahorice iznosila je 120 kg ha^{-1}, a pšenice 0, 15, 20, 25 i 30 kg ha^{-1}. Oged je postavljen po randomiziranom blok sistemu u četiri ponavljanja, površina osnovne parcelice iznosila je 60 m^2. Kosidba useva obavljena je u fazi formiranja mahuna. Pokošena masa sušena je na parceli do sadržaja vlage od 55%. Pokošena i prosušena masa smeštena je u silotrenč bez dodatka inokulanta. Nakon 45 dana fermentacije uzeti su uzorci senaže od po 400 g. Obavljena je standardna analiza osnovnih pokazatelja kvaliteta (sadržaj sirovih proteina, sirovih masti, sirove celuloze, pepela i BEM) i analiza po Van Soest-u (NDF, ADF i lignin). Prosečan prinos senaže iznosio je 15733 kg ha^{-1}. Najmanji prinos ostvaren je na tretmanu bez pšenice, 12240 kg ha^{-1}, a najveći na tretmanu sa 30 kg ha^{-1} pšenice, 18938 kg ha^{-1}. Nisu utvrđene signifikantne razlike u prinosu senaže na tretmanima sa 15 i 20 kg ha^{-1} pšenice (14970 i 15366 kg ha^{-1}). Prosečan sadržaj sirovih proteina iznosio je 21,44%, najveći je bio na tretmanu bez pšenice 25,18%, a najmanji na tretmanu sa 30 kg ha^{-1} pšenice 15,82%. Povećanjem učešća strnine u smeši sa grahoricom značajno se smanjuje poleganje useva, pozitivno se utiče na prinos krme ali je proizvedena senaža slabijeg kvaliteta. Pokazatelji kvaliteta kao što su sadržaj NDF, ukupno svarljivih nutrijenata (TDN), konzumiranje suve materije senaže (DMI) i relativna hranljiva vrednost (RFV) bili su najveći u čistom usevu grahorice, kao i na tretmanu sa najmanjom setvenom normom pšenice. Nisu utvrđene statistički značajne razlike između tretmana za sadržaj ADF, svarljivost suve materije (DDM) i NE_L. Najpovoljniji odnos između prinosa i kvaliteta senaže, te najveći prinos sirovih proteina (1482 kg ha^{-1}) ostvaren

je mešavinom 120 kg ha^{-1} semena grahorice i 15 kg ha^{-1} semena pšenice, stoga ova kombinacija može da se preporuči proizvođačima za agroekološke uslove jugoistočne Evrope.

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CARCASS COMPOSITION AND CHEMICAL CHARACTERISTICS OF MEAT FROM BROILER CHICKENS REARED UNDER INTENSIVE AND SEMI-INTENSIVE SYSTEMS

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Abstract: Poultry rearing systems have captured the attention of scientists for many years. A number of attempts have been made to introduce new technologies in rearing poultry for meat and egg production aiming at improving rearing conditions, protecting the environment and enhancing the quality of poultry products. Given the above, the objective of this study was to present the results of an evaluation of the effect of rearing system on major meat quality traits (percent yield of primal cuts in cold carcasses, tissue percentage in major primal cuts and chemical composition of muscle tissue). The experiment involved a total of 400 Hybro broilers reared under two different systems (intensive and semi-intensive systems). The fattening period and slaughter and dressing procedures were followed by measurement and meat sampling for chemical analysis. The results obtained show that broilers reared under the semi-intensive system had a somewhat higher percentage of drumsticks and breasts i.e. first class meat, as well as a higher percentage of breast and thigh muscles. Moreover, the protein content of breast, thigh and drumstick muscles was higher in broilers reared under semi-intensive conditions. As for the fat content of primal cuts, it was found to exhibit higher values in broilers reared under the intensive system. Overall, the results suggest better performance for most traits in free range broilers reared under semi-intensive conditions as compared to those reared intensively.

Key words: broilers, rearing systems, meat quality.

Introduction

Modern poultry rearing tendencies in developed European countries necessitate definition of adequate innovative rearing technologies to meet the criteria regarding the production of biologically valuable food, animal protection and rationalization of the production process.

European Union regulations on non-commercial, ecological and organic production are quite strict.

The most important requirements include housing under sufficient daylight conditions, limited stocking density i.e. limited number of birds per unit area, use of slow-growing chickens, and free-range recommendations.

Concerns regarding nutrition and length of fattening period are separately defined *(Ristić, 2005)*.

The effect of rearing system on growth and meat quality of broilers has been studied by a number of authors *(Lewis, 1997; Ristić, 1999; Damme and Rychlik, 2001; Holcman, 2003; Bogosavljević-Bošković et al., 2006a, 2010 etc.)*. Results of previous research *(Bogosavljević-Bošković et al., 2006b, 2011)* have shown that broiler hybrids most commonly reared in Serbia for intensive purposes can be successfully reared under semi-intensive i.e. free-range conditions. Certain meat quality traits obtained under this rearing system have proved to be better as compared to those of intensively reared broilers.

Given the above, the objective of this study was to evaluate the significance of differences in major meat quality traits (percent yield of primal cuts, tissue percentage in major primal cuts, and chemical composition of muscle tissue) in broilers reared under intensive and semi-intensive systems.

Materials and Methods

This experiment involved a total of 400 day-old Hybro broilers. Previous studies *(Bogosavljević-Bošković et al., 2006b, 2011)* have revealed that this line of broilers can be successfully reared under free-range i.e. semi-intensive conditions; hence the authors deemed it justified to use this line in the present experiment as well.

Over an initial period of four weeks, the test broilers were reared indoor on deep litter floor. Then, on day 28 of age, the broilers were assigned to two groups: Group 1, including broilers reared indoor, and Group 2, comprising broilers that were provided a grass range of $1m^2$/bird. Length of the fattening period was 56 days. Broiler nutrition involved the use of complete compound feeds, including broiler starter diet (BS, manufacturer: Veterinary Institute Zemun, Republic of Serbia) until day 28, and broiler finisher diet (BF, the same manufacturer) until day 42. From day 42 until the end of the fattening period, the broilers were fed a diet containing a mixture of ground maize, barley, mineral and vitamin supplements (70%) and a complete compound feed (30%). Feed provision was on an ad libitum basis.

Upon fattening, 15 male and 15 female broilers were randomly selected from each experimental group and slaughtered to examine major meat quality traits. Immediately prior to slaughter, the broilers were weighed. Upon slaughter, dressed carcasses were subjected to measurements. Thereafter, dressed cold carcasses were dissected into primal cuts (breast, drumstick, thigh, wing, pelvis and

back) following the method prescribed by the Regulation on Poultry Meat Quality *(Official Gazette of the SFRY Nos. 1/81 and 51/88)*. Primal cuts were weighed to determine the dressing percentage of the test broilers. The data obtained were used to calculate the percentage of individual meat classes. Then, major primal cuts, including breasts, thighs and drumsticks, were dissected into muscle tissues to evaluate their respective percentage. Muscle tissue was weighed and sampled for chemical analysis. The samples were stored at -21 ºC until analysis performed within one month from the sampling date.

The data obtained in this study were subjected to conventional methods of variation statistics. The significance of differences was tested by the following mathematical model of analysis of variance:

$$Yijk= \mu+(RS)_i+(S)_j+(RSS)_{ij}+e_{ijk}$$

The outlined model matched the 2 x 2 factorial design (2 rearing systems - RS, and 2 sexes – S).

The test parameters underwent analysis of variance (ANOVA) using Microsoft STATISTICA Ver. 5.0., Stat Soft Inc. (2005).

All significant differences determined by analysis of variance and results of F-exp values were evaluated using LSD-test.

Results and Discussion

The percent yields of individual meat classes in dressed broiler carcasses are outlined in Table 1.

Table 1. Dressing percentage and proportion of individual meat classes (%)

Rearing system (RS) (1)	Sex (2)		Dressing percentage	Meat classes		
				I	II	III
Intensive	male	$\bar{x}$	65.44	59.70	13.04	26.58
		Cv	1.90	2.94	6.84	6.94
	female	$\bar{x}$	65.61	59.76	12.60	27.17
		Cv	2.04	3.52	5.12	7.54
Semi-intensive	male	$\bar{x}$	64.93	60.57	12.75	26.01
		Cv	1.72	2.76	5.47	6.25
	female	$\bar{x}$	65.33	59.96	12.40	27.01
		Cv	1.37	3.88	6.10	8.77
F_{exp}		F_1		1.093^{ns}	1.599^{ns}	0.514^{ns}
		F_2		0.266^{ns}	4.159^{*}	2.315^{ns}
		F_{12}		0.427^{ns}	0.044^{ns}	0.157^{ns}

$\bar{x}$ - Average, Cv -Index of variation, ^{ns} –P>0.05; * – 0.01≤P≤0.05; ** – P<0.01

Table 1 shows that the highest percentage of class 1 meat was obtained in male broilers reared under semi-intensive conditions (60.57%) and the lowest in males from intensive rearing (59.70%). Smaller differences were observed between female broilers from the two experimental groups. The differences exhibited in terms of the percentage of class 1 meat were found to be significant (P>0.05). The range of values for class 2 meat of 12.40% (female broilers, semi-intensive system) to 13.04% (male broilers, intensive system) and class 3 meat of 26.01% (males, semi-intensive rearing) to 27.01% (females, semi-intensive system) suggests small differences between the percentage of class 2 and class 3 meat. Moreover, the differences observed were not statistically significant (P>0.05).

The percent yield of muscle tissue in major primal cuts is an important meat quality parameter in broilers. Table 2 presents the results on the percent yield of muscle tissue in breast, thighs and drumsticks (classified as class 1 meat in the Regulation on Meat Quality – *Rašeta and Dakić, 1984*).

Table 2. Percent yield of muscle tissue (%)

Rearing system (RS) (1)	Sex (2)		Muscle tissue		
			Breasts	Drumsticks	Thighs
Intensive	male	$\bar{x}$	65.78	59.11	69.28
		Cv	2.30	4.23	3.18
	female	$\bar{x}$	67.49	59.26	69.34
		Cv	3.09	0.86	3.17
Semi-intensive	male	$\bar{x}$	68.28	57.24	72.36
		Cv	1.65	3.34	0.71
	female	$\bar{x}$	69.85	58.25	72.66
		Cv	2.09	0.81	1.34
F_{exp}		F_1	7.902^{*}	2.262^{ns}	13.344^{**}
		F_2	3.627^{ns}	0.344^{ns}	0.045^{ns}
		F_{12}	0.004^{ns}	0.199^{ns}	0.019^{ns}

$\bar{x}$ - Average, Cv -Index of variation, ^{ns} –P>0.05; * – 0.01≤P≤0.05; ** – P<0.01

Table 2 shows that the percent yield of breast muscle tissue was higher in female broilers as compared to males, as well as in free-range broilers as compared to intensively reared broilers. However, Group 1 broilers had a higher percent yield of drumstick muscle tissue (59.11% and 59.26% in males and females, respectively). Semi-intensive rearing resulted in the percent yield of 57.24% (males) and 58.25% (females). The highest thigh content of muscle tissue (72.66%) was produced in free-range semi-intensive female broilers and the lowest (69.28%) in broilers reared under intensive system.

Differences in the percent yield of breast muscle tissue and drumstick muscle tissue were found to be statistically significant ($P<0.05$) and very significant ($P<0.01$) in favour of semi-intensive broilers.

Regardless of a number of factors that hinder comparison with the literature data, the results of the present research are in agreement with those reported by *Lewis et al. (1997), Ristić et al. (2003), Bogosavljević-Bošković et al. (2006)*.

The results on the protein and lipid contents in breast, drumstick and thigh muscles are presented in Table 3.

Table 3. Percentage of proteins and lipids in major primal carcass parts (%)

Rearing system (RS) (1)	Sex (2)		Breasts		Drumsticks		Thighs	
			Proteins	Lipids	Proteins	Lipids	Proteins	Lipids
Intensive	male	$\bar{x}$	23.48	2.10	21.88	4.46	19.96	7.92
		Cv	2.02	7.82	0.84	2.64	2.12	3.44
	female	$\bar{x}$	23.16	2.34	19.96	6.04	18.88	9.20
		Cv	1.82	3.08	0.80	3.75	1.22	2.26
Semi-intensive	male	$\bar{x}$	23.82	1.92	22.12	3.66	19.98	7.06
		Cv	1.07	3.31	0.92	4.52	1.27	3.54
	female	$\bar{x}$	23.48	2.16	21.72	3.48	19.02	7.84
		Cv	1.39	1.93	1.04	2.64	1.43	6.39
F_{exp}	F_1		21.679^{**}	57.940^{**}	178.48^{**}	24.605^{**}	24.244^{**}	134.67^{**}
	F_2		0.507^{ns}	0.00^{ns}	3.482^{ns}	1.232^{ns}	0.023^{ns}	1.603^{ns}
	F_{12}		0.010^{ns}	0.019^{ns}	0.007^{ns}	8.441^{**}	1.030^{ns}	1.113^{ns}

$\bar{x}$ - Average, Cv -Index of variation, ^{ns} –$P>0.05$; * – $0.01\leq P\leq 0.05$; ** – $P<0.01$

Table 3 shows that the protein content of breast muscles ranged from 23.16% (females, semi-intensive system) to 23.82% (males, semi-intensive system), and the lipid content from 1.92% (males, semi-intensive system) to 2.34% (females, intensive system). Both male and female broilers reared under semi-intensive conditions had a somewhat higher protein content and a lower lipid content in breast muscles. The protein content of drumsticks was also higher, and the fat content was lower in free-range broilers than in intensively reared broilers.

Broiler thighs had a protein content of 19.98% (males, semi-intensive system) to 18.88% (males, intensive system), and a lipid content of 7.06% (males, semi-intensive fattening) to 9.20% (females, intensive fattening).

Overall, irrespective of relatively small differences, semi-intensive rearing involving access to free-range conditions resulted in a higher protein content and a lower lipid content in all major primal cuts of broiler carcass as compared to intensively reared broilers.

The higher protein content of breast, drumsticks and thighs in broilers from semi-intensive system as compared to those reared under intensive conditions was confirmed by statistical analysis to show very significant differences ($P<0.01$).
The published data show substantial variation in the nutritional value of chicken meat. The present results on the protein and fat contents of white meat (breast muscle) and dark meat (leg muscle) complied with the findings obtained by *Demby and Cunningham (1980).* These authors reported the protein content of raw chicken meat of 17.0% to 23.3% (averaging 19.8%) and the fat content of 1.0% to 17.45% (average 7.5%). However, many variables, such as broiler hybrid or breed, age, sex, nutrition, rearing system, carcass dressing and type of meat, which can affect the nutritional value of meat can also induce small or large differences in the results obtained. High significant variability in terms of the fat content can also be due to the use of different sampling methods. Different authors have determined fat content either in muscle alone or in muscle-plus-skin, with subcutaneous fat being also included. Breast muscle-plus-skin was found by *Holcman et al. (2003)* to contain 20.4% protein and 7.0% fat on average. As for leg muscle-plus-skin, the average values as determined by the same authors were as follows: 16.8% protein and 13.1% fat.

Žlender et al. (1995) evaluated five different indoor-reared genotypes and reported the protein content of leg muscle and breast muscle-plus-skin to range from 15.8% to 17.9% and from 21.9% to 23.5%, respectively. The fat content of thigh muscle ranged from 10.6% to 15.6% and that of breast muscle from 3.9% to 8.45%.

Grashorn and Brose (1997) underlined that different production systems result in different meat quality. However, they reported higher differences only for extensive indoor broilers fattened according to the environmentally sound and controlled production standards, as opposed to label broilers (less intensive rearing conducted following the label concept) which produced meat quality similar to that obtained by broilers from conventional commercial production system. *Muriel and Pascual (1995)* found no significant differences in the protein content of muscles in indoor-reared male broilers slaughtered on day 85 of age and free range males slaughtered on day 81. Indoor-reared broilers had a somewhat lower meat protein content as compared to free range broilers. *Holcman et al. (2003)* reported that the chemical composition of breast and leg muscle-plus-skin in broilers aged 56 days is not affected by fattening system employed in compliance with the regulations of the European Union on “extensive indoor“ and “free range“ production systems. Conversely, in his study on meat quality of five different broiler genotypes reared under ecological conditions, *Ristić (2003)* observed the effect of production system on the chemical composition of breast and drumstick meat. The results of our research *(Bogosavljević-Bošković et al. 2004)* also suggest the effect of rearing system and broiler sex on the protein and fat contents of breast and leg muscles. The above results can be attributed to the fact that extensive indoor rearing system and free range system involving access to natural environmental conditions (fresh air and sunlight) also resulted in differences in terms of the structural

manifestations of tissues and organs, as well as in terms of the biochemical processes involved in the metabolism.

Conclusion

The results of the present study on the percentage of certain meat classes, percent yield of muscle tissue in breasts, thighs and drumsticks and their respective protein and fat contents suggest the following:

- No statistically significant differences in the percentage of class I, class II and class III meat were observed between rearing systems and broiler sex ($P>0.05$).
- Broilers reared under semi-intensive conditions had a higher percent yield of muscle tissue in breasts (statistically significant differences, $P<0.05$), drumsticks (non-significant differences, $P>0.05$) and thighs (statistically very significant differences, $P<0.01$) as compared to broilers from intensive rearing.
- The protein content of breast, drumsticks and thighs was higher, and the lipid content was found to be lower in semi-intensively reared broilers as compared to intensively produced broilers, with the differences observed being statistically very significant ($P<0.01$).

Semi-intensive broiler rearing system was found to have an advantage over intensive rearing system in terms of both the percent yield of muscle tissue in breasts, drumsticks and thighs and the chemical composition of muscle tissue of class 1 meat.

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Kompozicija trupova i hemijski sastav mesa tovnih pilića iz intenzivnog i poluintenzivnog načina gajenja

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Rezime

Sistemi gajenja živine već duži niz godina u mnogim zemljama sveta zaokupljaju pažnju i nauke i struke. Čine se brojni pokušaji da se u gajenju živine namenjene proizvodnji mesa i jaja inoviraju i ustanove nove tehnologije koje bi

doprinele, pre svega, poboljšanju uslova gajenja, zaštiti životne okoline i koji bi osigurali bolji kvalitet živinskih proizvoda.

Polazeći od navedenog, cilj ovog rada bio je da se prikažu rezultati ispitivanja uticaja sistema gajenja na važnije osobine kvaliteta mesa (udeo osnovnih delova u obrađenim trupovima, udeo tkiva u važnijim osnovnim delovima i hemijski sastav mišićnog tkiva). Za istraživanje je poslužilo ukupno 400 pilića linijskog hibrida Hybro koji su gajeni na 2 različita načina (intenzivan i poluintenzivan). Po završetku tova i nakon klanja i primarne obrade trupova izvršena su neophodna merenja i uzeti su uzorci za analize hemijskog sastava mesa.

Rezultati ovog istraživanja pokazuju da su pilići odgajani na poluintenzivan način imali nešto veći udeo bataka i grudi, tj. mesa I kategorije, kao i veći udeo mišićnog tkiva u grudima i karabatacima. Osim navedenog, sadržaj proteina u mišićnom tkivu grudi, bataka i karabataka bio je veći kod pilića iz poluintenzivnog načina gajenja. S druge strane, sadržaj masti u navedenim osnovnim delovima bio je veći kod pilića iz intenzivnog načina gajenja.

U celini posmatrano, rezultati ovih istraživanja pokazali su da su pilići gajeni uz korišćenje ispusta tj. na poluintenzivan način u većini ispitivanih osobina postigli bolje rezultate u odnosu na piliće iz intenzivnog načina gajenja.

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THE EFFECT OF MODIFICATION OF INCUBATION FACTORS ON THE QUALITY OF BROILER CHICKENS MEAT

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Abstract: Embryonic stage of development, in conditions of intensive broiler production, is a period in which the dynamics of postnatal development and the productive performance can be affected significantly. Green monochromatic light and thermal conditioning are the most commonly used treatments that have given satisfactory results in postnatal development. The aim of this study was to examine the effect of these treatments on meat quality of broiler chickens. Our study is based on the four experimental groups (control and three different treatments). Each group was incubated in separate incubator with 80 eggs, with controlled incubation parameters (temperature, humidity, ventilation). The control group was incubated under standard conditions (37.8 ˚C, without light). The first treatment consisted of thermal conditioning (raising the temperature to 39,8 ˚C for 3 h on 16th, 17th and 18th day of incubation). Another treatment consisted of lighting a green monochrome light (diffused LED lighting intensity of 0.1 W/m2) from 5th until 14th day of incubation, in the intermittent mode (15 min. light, 15 min. darkness), and from 15th day of incubation under constant light regime. The third treatment was a combination of first two treatments, with the temperature and lighting changed by these regimes. Our results indicate a significantly higher average body weight, protein content in meat, and less progressive loss of moisture (drip loss) in all treated groups. The applied treatments had no effect on mortality, conversion, or the content of fat and ash in meat. The results also indicate a significant synergistic effects achieved by the simultaneous application of both treatments.

Key words: incubation factors, heavy hybrids chickens, meat quality

Introduction

There are several ways to affect, during embryonic development, the stimulation of muscle growth, and thereby the production of meat. The literature describes methods for achieving these results based on the application of

monochromatic green light and thermal conditioning (temperature increase during the incubation in certain critical stages) during embryonic development or in early postnatal development *(Halevy et al., 2006)*.

Light can affect muscle tissue directly or indirectly. Direct effect is reflected in the penetration of monochromatic green light through the egg shell and affecting the muscle tissue directly. Alternatively, light can affect the myogenesis indirectly through the endocrine system or through extra cellular mitogens produced in adjacent tissues. Light energy can reach the hypothalamus directly through the skull and soft tissues *(Lewis and Morris, 2000)*.

Increased expression of mRNA (messenger ribonucleic acid) for receptors for growth hormone was detected in satellite cells, derived from the chickens that were treated with a green monochrome light *(Halevy et al., 1998)*. Growth hormone has been marked as a mitogen of satellite cells, regulating the number of receptors on these cells by positive feedback *(Sadowwski et al., 2001)*. Indirect effects of growth hormone on the muscle tissue can be achieved through triiodothyronine. High levels of growth hormone in the circulation, before hatching, affects the increased thyroxine monodeiodination in triiodothyronine in the liver of birds *(Darras et al., 1990)*.

Studies suggest that thermal conditioning at an early age, leads to temporary halt in growth that is followed with increased compensatory growth. This process leads to the body and breast muscle weight gain in treated broiler chickens in compare to the control group, at a later age *(Yahav, 2000)*. Recent studies show that this treatment has a stimulatory effect on the growth of skeletal muscle through the stimulation of proliferation of satellite cells and their accelerated differentiation *(Halevy et al., 2001)*.

The main mediator of responses of satellite cells, exposure to moderately increased temperature, are locally produced growth factors, primarily those produced in the muscles themselves. This is indicated by increased expression of IGF-1 (insulin-like growth factor 1) protein in breast muscle of chickens subjected to such treatment, with an accompanying proliferation of satellite cells. It is proved that IGF-1 stimulates proliferation of primary satellite cells in chicken *(Halevy et al., 2001)*.

Based on data from the literature, we set the research goal to determine the influence of the monochromatic green light and thermal conditioning, and their combined action, during embryonic development, on the meat quality of the broilers chickens. In addition to this we wanted to give a general assessment of the justification of their use in commercial broiler production.

Materials and Methods

For this trial we have used the fertilized eggs of heavy hybrid "Ross 308", originating from parent flock at the age of 54 weeks. The average egg weight was 66.5 g, while egg weight ranged from 63.5 g to 69 g.

Temperature was regulated using an electronic thermostat "*Thermoregulator ET-01*" producers "*Pro-Electric*", Novi Sad, Serbia. Monochromatic green light was achieved using LED (light emitting diode) lighting, using "*JCDR LED 18*" (1.2 W 10000-12000 mcd) light source, manufactured by "Vito Industrial Limted, Zhuhai, China. Light intensity and homogeneity of light were controlled with luxmetr "*Peak Teck 5025*", produced by "*Peak Technologies, LLC*." Old Columbia Rd., Columbia, USA.

The experimental groups (Eg) were formed according to the following principles:

EgA was the control group that was incubated under standard conditions (constant temperature of 37.9 ˚C, without light, with relative humidity and ventilation that were adapted to the respective stages of embryonic development);

EgB was incubated under standard conditions until the 15th day of embryonic development. During 16^{th}, 17^{th} and 18^{th} day of the embryonic development the temperature was increased by 1.5 ˚ C for 3 hours (from 09:00 h to 12:00 h). From 19th day until the end of incubation, the incubation factors were identical as in the EgA.

EgC was incubated under standard conditions untilo 5^{th} day of embryonic development. From 6th to 15^{th} day of embryonic development it was subjected to the influence of monochromatic green light (wavelengths from 450 to 550 nm and intensity 0.1 W / m^2) under intermittent regimen of 15 minutes of light and 15 minutes of darkness. After that period, from 16^{th} day of incubation, until the end of the embryonic development the light was constant.

EgD was a combination of experimental treatments EgB and EgC.

After hatching, drying and rest for 24 hours, broiler chickens were packed into transport boxes, separated on experimental groups and transferred to the experimental farm of Faculty of Agriculture in Novi Sad "*Pustara*" in Temerin. Chickens were vaccinated using a regular vaccination program and throughout the trial were under constant veterinary supervision. During the production cycle the broilers were fed ad libitum in three phases using a complete feed mixture (CFM) adjusted for their age.

The measurement of body weight was carried out using the scales "Mettler K5", produced by "*E. Mettler*", Zurich, Switzerland. The moisture content in meat samples was determined by drying at 105 ˚C to constant weight. The percentage of protein in meat was determined by Kjeldahl method. The percentage of fat in meat was determined by Soxlet method. Ash content in meat was determined by annealing, or heating the sample at 500 ˚C. The progressive loss of moisture from the brest muscles ("Drip loss") was determined by measuring the weight of the samples of brest muscles before and after storage at +4 ˚C for 24 and 48 hours, as described in *Perić et al. (2009)*.

The degree of statistical significance between groups was performed by analysis of variance (ANOVA) and Student's t-test, for the significance level of

95% 95% ($p<0,05$) and 99% ($p<0,01$). The calculation was performed using PC and *Microsoft Excel 2003*.

Results and Discussion

The results related to the basic production parameters (body weight, mortality and conversion) are shown in Table 1.

Table 1 Basic production parameters

Parameter	EgA	EgB	EgC	EgD
Body weight (g)	$2089^{b,c,d}$	2243^{a}	2222^{a}	2272^{a}
Mortality (%)	3,0	2,0	3,0	2,0
Conversion	1,911	1,887	1,908	1,906

^{a,b,c,d} – statistically significant difference ($p<0,05$)
^{A,B,C,D} – statistically very significant difference ($p<0,01$)

Monochromatic green light shows the stimulating effect on growth and this effect can be expected in the embryonic stage of development *(Rosenboom et al., 2004)*. Thermal conditioning results in increased body weight *(Yahav, 2000)*. Application of light of different wavelengths had no effect on conversion and mortality *(Wabeck and Skoglund, 1974)*. Our study showed a statistically significantly higher body weight in all treated groups than in the control group, and no impact on mortality and conversion. The main parameters of meat quality (dry matter content, chemical composition and progressive loss of moisture) are shown in Table 2

Table 2 Meat quality parameters

Parameter	OgA	OgB	OgC	OgA
Dry matter content (%)	$27,74^{B,C,D}$	$30,17^{A}$	$30,17^{A}$	$30,94^{A}$
Protein content (%)	$18,70^{B,C,D}$	$19,94^{A}$	$19,45^{A}$	$19,56^{A}$
Lipid content (%)	2,05	2,24	1,88	1,96
Ash content (%)	1,02	1,04	1,05	1,03
Progressive loss of moisture after 24 h (%)	$1,88^{b,c,d}$	$1,11^{a}$	$1,26^{a}$	$1,20^{a}$
Progressive loss of moisture after 48 h (%)	$1,97^{b,c,D}$	$1,43^{a}$	$1,56^{a,D}$	$1,36^{A,C}$

^{a,b,c,d} – statistically significant difference ($p<0,05$)
^{A,B,C,D} – statistically very significant difference ($p<0,01$)

Alvarado and Owens (2006) report that the percentage of protein in muscle tissue of broilers was 19.14%. *Lesiów (2006)* finds that the total fat content in muscle tissue varies considerably from 1,58 % to 6,65 %. *Ristic et al. (2007)* reported that the percentage of ash in broiler meat makes an average of 1.2% by weight fresh meat after slaughter.

The results show that all treated experimental groups had statistically significantly higher dry matter content and lower moisture content in muscle, in compared to the control experimental group. These results indicate that the treatment, which influenced the increase in total body weight, acting on account of higher dry matter content and not on account of higher moisture content in meat. The increase in the percentage of dry matter in the meat generally can result with the increase or decrease its nutritional value, depending on whether it is achieved at the expense of increasing the total content of protein or fat as the main nutrients. Our results indicate that the percentage of protein was significantly higher in all treated groups compared to the control group, and ranged above 19%, while in the control group it was below this value. In addition, our results indicate the absence of statistically significant differences between all experimental groups in the lipid and ash content in meat.

The progressive loss of moisture is an important indicator of meat quality, since the weight loss increases during the technological process of cooling. This moisture loss is greatest in the early days, so it is common that it is expressed as the percentage mass loss after 24 and 48 hours of cooling. The moisture loss is related primarily to the loss of free water that is free in the cell or the extra cellular space. Our results show, unequivocally, the beneficial effect of the treatments on the progressive loss of moisture from the meat after 24 hours and 48 hours of cooling.

Conclusion

All the applied treatments show significant results in increasing body weight, without affecting mortality and conversion. Treatments have a significant impact on the increase of dry matter in the meat which is a result of increasing the percentage of total proteins, while treatments have no effect on fat and ash content in meat. All treatments have a significant effect on reducing the progressive loss of moisture from the meat.

Based on these results we can conclude that it is reasonable to use treatments that are based on the modification of incubation factors, and that there is a possibility that they can be successfully implemented in commercial broiler production.

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Uticaj modifikovanih inkubacionih faktora na kvalitet mesa brojlera

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Rezime

Faza embrionalnog razvoja u uslovima intenzivne brojlerske proizvodnje je period koji značajno utiče na postnatalni razvoj i proizvodne performance. Zelena monohromatska svetlost i temperaturni uslovi su najčešće upotrebljavani tretmani koji daju zadovoljavajuće rezultate u postnatalnom razvoju. Cilj ovog rada je ispitivanje uticaja ovih tretmana na kvalitet mesa brojlera.

Naša istraživanja su bazirana na četiri eksperimentalne grupe (kontrola i tri različita tretmana). Svaka grupa je inkubirana u odvojenom inkubatoru sa 80 jaja, sa kontrolisanim inkubacionim periodima (temperatura, vlažnost, ventilacija). Kontrolna grupa je inkubirana pod standardnim uslovima (37.8 ˚C, bez svetla). Prvi tretman se odnosio na temperaturne uslove (povećanja temperature tokom 3 h do 39,8˚C, 16., 17., 18. dana inkubacije). Drugi tretman je uključivao upotrebu zelene monohromatske svetlosti (difuzne LED svetlosti intenziteta 0.1 W/m^2) od 5. do 14. dana inkubacije, u naizmeničnim intervalima (15 min svetla, 15 min mraka), i od 15. dana inkubacije konstantan svetlosni režim. Treći tretman je bio kombinacija prva dva tretmana sa temperaturnim i svetlosnim izmenama u datim režimima.

Naši rezultati ukazuju na značajno veće prosečne telesne mase, sadržaj proteina u mesu i manje progresivan gubitak vlage (drip loss) u svim tretmanima. Primenjeni tretmani nisu imali efekta na mortalitet, konverziju hrane i sadržaj mast i pepela u mesu. Rezultati takođe ukazuju na značajne sinergističke efekte koji su postignuti simultanom aplikacijom oba tretmana.

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THE INFLUENCE OF LONG TERM SOUND STRESS ON HISTOLOGICAL STRUCTURE OF BROILER'S ADRENAL GLANDS

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Abstract: The aim of this paper is to examine the effect of different sound stress duration on adrenal gland of broiler chickens of different age. The experiments were conducted on 90 HYBRO broiler chickens, divided into nine groups (each group consisting of 10 birds) by different age and lenght of sound stress duration. The chickens were exposed to sound stress in sound attenuated building using a fire alarm bell (95 dB) for stress sessions lasted 120 min every day. The histological structure of adrenal gland was analyzed by light microscopy. The results indicate that adrenal gland is sensitive on this kind of stress and sound as stressor could introduce organism in stress reaction. Adrenal glands of broilers exposed to sound stress characterized by marked hyperemia, hypertrophy of interrenal cells, smaller affinity for staining of chromaffin cells compared with control groups. According to our results, degree of histological changes of adrenal gland under the influence of sound stress depends on the length of exposure and age of chickens.

Key words: sound stress, broiler chickens, adrenal gland

Introduction

Stress is the reaction of the organism to stimulus which disturbe physiological equilibrium, usually with harmful consequences. This disturbance of homeostasis results in changes in the concentration of large number of different hormones that have a crucial role in the regulation of immune system function.

There is experimental evidence that noise exposure may be a potential stressor in farm animal husbandry. Physiological and behavioural studies have identified noise stress during housing *(Talling et al., 1998a; Schaffer et al., 2001)*, transport and at the abattoir *(Geverink et al., 1998)*. Exposure to noise stress is

known to activate the HPA axis in different species *(Engeland et al., 1990; Van Raaij et al., 1997; Evans et al., 2001; Monsefi et al., 2006)*.

Noise as a stress factor in fowls has not yet been sufficiently investigated. According to *Campo et al. (2005)* noise seems to affect adversely the productive performance and behaviour of the birds. *Stadelman (1958a)* reported violent behavioural response in young chickens to intermittent sound exposure at 100-118 dB. Sound intensities of 115 dB were effective in interrupting brooding in hens *(Stadelman, 1958b)*. A significant negative effect of acute noise exposure at 80 dB and 100 dB on stressfulness in broilers was described by *Chloupek et al. (2009)*, who found a significant increase in corticosterone and cholesterol levels after 10 min of noise exposure.

Changes in structure of adrenal gland in poultry, as results of influence of diffrent stressors, indicate that adrenal glands are involved in stress reaction *(Nir et al., 1975; Carsia and Weber, 1986; Mahata et al., 1990, Cheng and Muir, 2004, Moneva et al., 2009)*.

The aim of our investigation was to estimate the influence of long term sound stress on adrenal gland morphology in broiler chickens.

Materials and Methods

The experiments were conducted on 90 HYBRO broiler chickens, divided into nine groups (each group consisting of 10 birds) as follows:

$C_{1\text{-}15}$ - control (non exposed) group
$O_{1\text{-}15}$ - chickens exposed to the sound stress from 1st to 15th day of life
$C_{1\text{-}30}$ - control (non exposed) group
$O_{1\text{-}30}$ - chickens exposed to the sound stress from 1st to 30th day of life
$O_{15\text{-}30}$ - chickens exposed to the sound stress from 15th to 30th day of life
$C_{1\text{-}45}$ - control (non exposed) group
$O_{1\text{-}45}$ - chickens exposed to the sound stress from 1st to 45th day of life
$O_{15\text{-}45}$ - chickens exposed to the sound stress from 15th to 45th day of life
$O_{30\text{-}45}$ - chickens exposed to the sound stress from 30th to 45th day of life

The control and exposed chickens were kept in two different buildings under same conditions of temperature, light, humidity and number of birds per m^2. They were fed ad libitum and had free access to water. According to the experimental schedule, chickens were moved from the building without noise to the building where they were subjected to sound stress. The chickens were exposed to sound stress in sound attenuated building using a fire alarm bell (95 dB) for stress sessions lasted 120 min. every day. The bell was programmed to ring for 5 sec in a variable interval schedule (5 to 115s) through signals generated by PC software. Every 15 day, birds were sacrificed as show in above schedule. For histological investigation samples of adrenal glands were fixed in Bouin solution

for 24 h and after standard histological procedure of dehydration organs are were embedded in paraffin. Serial cuts, 5 μm thickness, were made by microtome and stained with hematoxyline and eosine. Histological analysis was performed with light microscope Leica DLMS connected with camera (Leica DC-300). Software for image analysis was IM 1000 (Leica Imaging Systems Ltd, Cambridge, UK).

Results and Discussion

In chickens, adrenal glands may be a single median structure or two separate organs, always in close contact with each other, next to the kidneys. The gland is surrounded by a loose connective capsule and in contrast to mammals, a distinct steroidogenic cortex and a chromaffin medulla are not present. Rather, clumps or strands of chromaffin cells are admixed with blood vessels and interrenal steroidogenic cords, radially arranged, both in the subcapsular zone and in the inner part of the gland

The typical changes of adrenal glands between control groups and groups of chicken exposed to sound stress were showed at figures 1-3.

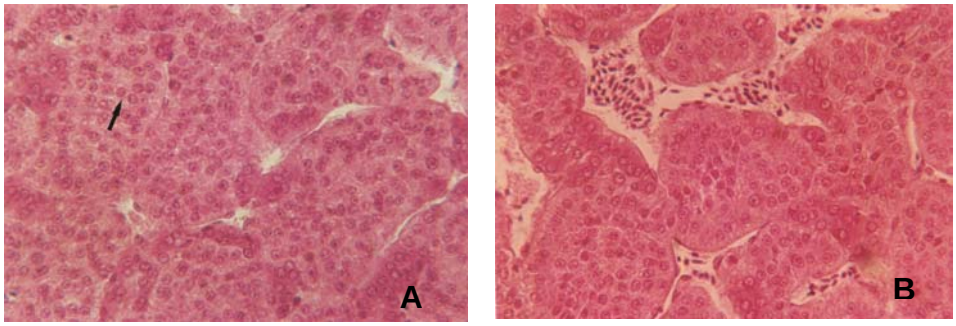

Figure 1. Microphotographs of chicken adrenal gland (A – control group, 15 days old; B – sound stress from 1st. to 15th days; arrow – interrenal cells (HE; 252x)

Adrenal glands of control group, 15th day old, heve clear diferentiation on interrenal and chromaffin cells, as well developed capillary network. Interrenal cells are grouped and with circular orientation as glomerul, while medulocites are localized near cappilary in formation of few cells (Figure 1A). In interrenal cells are evident good vacuolisation with significant lipid accumulation. In chickens exposed to sound stress at 15t day old, dilatation of blood vessels are marked (figure 1B). Interrenal cells of chickens exposured to sound stres from 1st to 15th day, characterized hyperthrophy with decreased vaculization and changes in cells size and shape. Chromaffin cells of exposed grupe have smaller afinity to staining then control group.

At the age of 30th day, adrenal gland of chickens from control group characterized clear diferentitation on interenal and chromaffin cells. In that age, light interrenal cells are dominant, but compared with control group of 15th day old, huge number of chromaffin cells exists (Figure 2A). In the group which was exposured to stress from 15th to 30th day, large number of dark interrenal, smaller diameter of cells and changes in nuclei, as well as individual very large light cells with merked vacuolization was observed (Figure 2B).

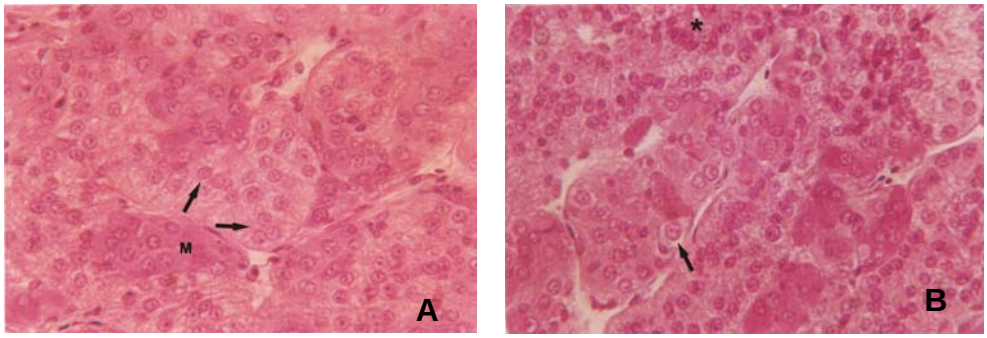

Figure 2. Microphotographs of chicken adrenal gland (A – control group, 30 days old; B – sound stress from 15th to 30th days; arrow – light interrenal cells; * - dark interrenal cells; M- chromaffin cells (HE; 400x)

The structure of adrenal gland of control group at 45th day of age is simular as in control groups at 15th and 30th day old chickens. In the group was exposured to stress from 15th to 45th days clear differet between light and dark interrenal cells. Light cellc are large with marked vacuolization, and dark are smaller, with less vacuoles and cytoplasm. At the chickens from that group large difference in diameter of cells and lagre number of cells with degenerative changes were visible (Figure 3).

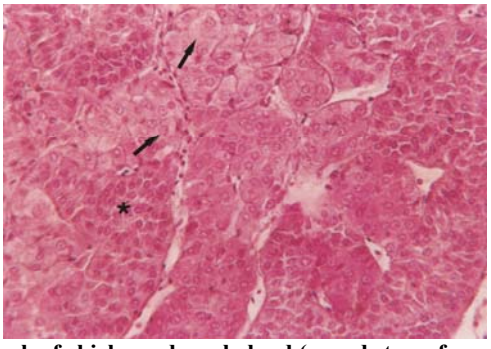

Figure 3. Microphotograph of chicken adrenal gland (sound stress from 15th to 45th days; arrow – light interrenal cells; * - dark interrenal cells (HE; 252x)

The results presented in this paper indicate the stressful effects of noise, and considering that the study of histological structure of adrenal gland becomes more important. Degree of histological changes of adrenal gland under the influence of sound stress depends on the length of exposure and age of chickens. Results about level of corticosterone in broiler chickens exposured to sound, according with our results, point out on stres reaction *(Bedanova et al., 2010)*. Also, our results *(Lazarevic et al., 2000)*, showed that sound have significant influence on change heterophil/lymphocyte ratio, which is one of imoprtant indicator of avian stress reaction *(McFarlane and Curtis, 1989)*. According with our result, adrenal glends of birds are known to respond to stress condition by interrenal hyperplasia *(Garren and Barber, 1955; Siegel, 1959; Feeman, 1970; Aire, 1980)* and changes in interrenal cord and interrenal and chromafine ratio *(Sharma et al., 2009)*.

Conclusion

Chronic sound stress causing significant changes in histological structure of adrenal gland in broiler chickens. These changes are reflected as hyperemia and changes in interrenal cells and chromaffin cells. The typical changes were result of stressogenic effect of sound, and these reactions point out that those chickens were in stress reaction.

Degree of histological changes of adrenal gland under the influence of sound stress depends on the length of exposure and age of chickens.

Uticaj dugotrajnog zvučnog stresa na histološku građu nadbubrežne žlezde brojlera

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Rezime

Cilj ovog rada je da ispita uticaj različite dužine trajanja zvučnog stresa na histološku građu nadbubrežnih žlezda brojlerskih pilića u različitim uzrastima. Ogled je izveden na 90 HYBRO brojlerskih pilića, podeljenih u 9 grupa u skladu sa uzrastom i dužinom izlaganja stresu. Pilići su zvučnom stresu bili izlagani u zvučno izolovanim objektima uz korišćenje požarnog alarmnog zvona (95dB) u trajanju od 120 min svakog dana. Histološka građa nadbubrežnih žlezda je analizirana svetlosnim mikroskopom. Rezultati ukazuju da je nadbubrežna žlezda osetljiva na ovu vrstu stresa i da zvuk, kao stresor, može da uvede organizam u stresnu reakciju. Nadbubrežne žlezde pilića izlaganih zvučnom stresu karakteriše

značajna hiperemija, hipertrofija interrenalnih ćelija, manji afinitet prema bojenju hromafinih ćelija u odnosu na kontrolnu grupu. U skladu sa ovim rezultatima, stepen histoloških promena nadbubrežne žlezde pod uticajem zvučnog stresa zavisi i od dužine izlaganja i uzrasta pilića.

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INFLUENCE OF MODIFIED INCUBATION FACTORS ON CARCASS CHARACTERISTICS OF BROILER CHICKENS

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Abstract: Incubation factors have a great influence on embryonic and postnatal development of broiler chickens. The aim of changing of incubation factors is to obtain the best possible production characteristics of broilers. In previous studies, it was determined that the application of monochromatic green light and thermal conditioning during incubation give the most satisfactory results on the postnatal development of chickens. This paper examines the effects of green monochromatic light and thermal conditioning during incubation on the carcass characteristics of broilers. In this paper four experimental groups were set. The first group was incubated under the standard conditions (37.8 ° C, no light). In the second group incubation temperature were increased, while in the third group incubation was carried out under green monochromatic light. In the fourth group temperature and light conditions of incubation were changed simultaneously. In all groups, changes of incubation factors were made at precisely defined time intervals. After hatching, chickens were grown up to day 42, when they were sacrificed and their carcass characteristics examined. Analysis of results showed that all treated groups have significant higher body weight, weight of drumsticks, thighs and toes. The fourth group have significant higher weight of breasts and wings compared to control group. These results are explained by the influence of the temperature and light changes during incubation on the increased proliferation of myoblasts, the growth of skeletal muscle cells, and the activity of satellite cells, which are reflected in changes of carcass characteristics.

Key words: broiler chickens, carcass characteristics, incubation factors

Introduction

Previous research showed that changing of light and temperature conditions of incubation had a significant effect on embryonic and postnatal

development of chickens, and their carcass characteristics as well *(Halevy et al., 2006c)*. It was found that green monochromatic light and narrow variation in temperature influences the development of skeletal musculature of chickens *(Andrews and Zimmerman, 1990; Halevy et al., 2006b)*.

Treatments of high temperature and green monochromatic light during incubation results in increased proliferation of myoblasts, as well as in increased development of satellite cells, which causes changes in the development of skeletal musculature *(Yahav et al., 2004; Rozenboim et al., 2004)*. Such changes are certainly reflected in the carcass characteristics of broilers *(Halevy et al., 2006c)*.

The aim of this study was to explore the effect of altered incubation factors (high temperature and green monochromatic light) on carcass characteristics of broilers.

Materials and Methods

In the experimental part of this paper, eggs that were incubated were divided into 4 groups (A, B, C, D). Incubation of eggs is carried out in incubators for 100 eggs (Phenyl, Beska, Serbia).

Group A was incubated under standard incubation conditions. In group B temperature conditions were changed (temperature of 39°C for 3 hours during 16th, 17th and 18th day of incubation). In group C from 6th day of incubation eggs were incubated under the influence of monochromatic green light, in a way that from 6th to 15th day of incubation intermittent light regime were applied (15 minutes of light, 15 minutes of darkness), and from 16th day of incubation eggs were exposed to constant light. Group D was incubated under the modified both light and temperature conditions, such as temperature change by the mode of group B, and light change by the mode of group C.

Temperature and humidity were controlled using analog polymeter ("Web", Berlin, Deutschland). Monochromatic green light were obtained with LED (light-emitting diode) lighting, using "JCDR LED 18" light source ("Vito Industrial Limited", Zhuhai, China), which provided light of wavelength range of 535-585 nm, and the intensity of 6.21 lx. Light intensity and homogenity of light were controlled with digital luxmeter "Peak Teck 5025 ("Peak Technologies, INC", Columbia, USA).

After hatching, chicks were transferred to the farm, where they were bred to 42nd day of life, when their carcass characteristics were determined.

Results and Discussion

It was established that on 42nd day after hatching, chickens of group B, C and D have a significantly higher body weight, body weight after bleeding and body weight without entrails ($p < 0.01$) comparing to group A (Table 1).

Table 1. Body weight (g), body weight after bleedding (g) and body weight without entrails (g) of broilers on 42nd day after hatching ($\bar{x} \pm SD$)

Group	Body weight	Body weight after bleeding	Body weight without entrails
A	$2029,29 \pm 94,18^{A}$	$1947,86 \pm 81,08^{A}$	$1702,14 \pm 72,45^{A}$
B	$2243,57 \pm 142,06^{B}$	$2150 \pm 137,23^{B}$	$1886,43 \pm 131,08^{B}$
C	$2222,14 \pm 125,49^{B}$	$2129,29 \pm 123,17^{B}$	$1852,86 \pm 88,40^{B}$
D	$2272,14 \pm 119,12^{B}$	$2190 \pm 122,20^{B}$	$1948,57 \pm 116,39^{B}$

$^{A-B}$ Means within the same parameter, without same capital letter superscript differ significantly ($p < 0,01$).

It is established that chickens of group D have significantly higher ($p < 0,05$) breast weight compared to group A and C. Weight of drumsticks and thighs were significantly higher ($p < 0,01$) in the chickens of group B, C and D compared to group A. Weight of toes were significantly higher ($p < 0,05$) in the chickens of group B, C and D compared to group A. Wings weight were significantly higher ($p < 0,05$) in the chickens of group D compared to groups A, B and C (Table 2).

Table 2. Body weight with entrails (g), weight of entrails (g), breasts (g), drumsticks and thighs (g), toes (g), heads (g), wings (g) and backs (g) on 42nd day after hatching of chickens ($\bar{x} \pm SD$)

Weight of body parts	Group			
	A	B	C	D
Body weight with entrails	$1947,86 \pm 81,08^{A}$	$2150 \pm 137,23^{B}$	$2129,29 \pm 123,17^{B}$	$2190 \pm 122,20^{B}$
Entrails	$245,71 \pm 28,49$	$263,57 \pm 40,38$	$276,43 \pm 44,41$	$241,43 \pm 16,76$
Breasts	$469,29 \pm 20,70^{a}$	$500,00 \pm 67,02^{ab}$	$472,14 \pm 30,53^{a}$	$557,14 \pm 85,33^{b}$
Drumsticks and thighs	$371,43 \pm 38,70^{A}$	$442,86 \pm 34,98^{B}$	$432,86 \pm 29,13^{B}$	$450,71 \pm 42,66^{B}$
Toes	$75,00 \pm 12,25^{a}$	$96,43 \pm 12,49^{b}$	$91,43 \pm 8,52^{b}$	$99,29 \pm 8,38^{b}$
Heads	$68,57 \pm 7,48$	$63,57 \pm 5,56$	$62,14 \pm 9,51$	$63,57 \pm 6,90$
Wings	$161,43 \pm 25,61^{a}$	$174,29 \pm 12,39^{a}$	$176,43 \pm 14,35^{a}$	$192,86 \pm 9,51^{b}$
Backs	$329,29 \pm 19,02$	$352,86 \pm 68,55$	$346,43 \pm 58,79$	$347,86 \pm 37,95$

$^{a-b}$ Means within the same parameter, without same small letter superscript differ significantly ($p < 0,05$).

$^{A-B}$ Means within the same parameter, without same capital letter superscript differ significantly ($p < 0,01$).

It is established that percentage of breasts in relation to the body weight is significantly higher ($p < 0,05$) in the chickens of group D compared to group C. Percentage of drumsticks and thighs in relation to the body weight is significantly higher ($p < 0,05$) in the chickens of group B and D compared to group A. Percentage of heads in relation to the body weight is significantly higher ($p < 0,05$) in the chickens of group A compared to group B, C and D (Table 3).

Table 3. Percentage of weight of entrails (%), breasts (%), drumsticks and thighs (%), toes (%), heads (%), wings (%) and backs (%) in relation to the body weight on 42nd day after hatching of chickens ($\bar{x} \pm SD$)

Percentage of weight of body parts	Group			
	A	B	C	D
Entrails	12,11 ± 1,28	11,76 ± 1,72	12,39 ± 1,46	10,64 ± 0,70
Breasts	23,16 ± 1,41^{ac}	22,21 ± 1,65^{ac}	21,27 ± 1,36^{bc}	24,42 ± 2,59^{a}
Drumsticks and thighs	18,27 ± 1,29^{a}	19,73 ± 0,35^{bc}	19,49 ± 1,00^{ac}	19,80 ± 0,89^{bc}
Toes	3,68 ± 0,50	4,29 ± 0,42	4,13 ± 0,54	4,39 ± 0,54
Heads	3,39 ± 0,45^{a}	2,83 ± 0,16^{b}	2,80 ± 0,43^{b}	2,81 ± 0,40^{b}
Wings	7,94 ± 1,12	7,78 ± 0,60	7,98 ± 0,98	8,51 ± 0,71
Backs	16,25 ± 1,10	15,72 ± 2,75	15,56 ± 2,36	15,36 ± 1,97

$^{a-c}$ Means within the same parameter, without same small letter superscript differ significantly ($p < 0,05$).

The effect of modified conditions of incubation on improved productive caracteristics of adult chickens can be explained by influence of green monochromatic light and increased temperature on growth and differentiation of skeletal muscle cells *(Rozenboiom et al., 2004; Halevy et al., 2006b; Halevy et al., 2006c)*. The applied treatments affect on increased diameter of skeletal muscle cells in the postnatal period of development in the treated groups, which affects on increased weight of white meat, drumsticks, thighs and wings of chickens. Also, these treatments affect on the increased proliferation of myoblasts *(Collin et al., 2005; Halevy et al., 2006a; Piestun et al., 2009)*, resulting in increased number of cells within a muscle. A great number of cells cause greater muscle mass in later stages of postnatal development.

In previous studies it was established that these treatments have a stimulatory effect on the proliferation of satellite cells, which affects on increased growth and development of skeletal musculature in the postnatal period of development *(Halevy et al., 2006c; Piestun et al., 2009)*, which could also affect on the production parameters of broilers.

The achieved results of this study are similar with results of other authors. When chickens during the incubation were exposed to monochromatic green light the increase of the weight of *m. pectoralis superficialis (Halevy et al., 2006a)*, the weight of *m. supeficialis pectoralis* and *m. pectoralis profundus*, as well as the

body weight at 42nd day after hatching of chicks *(Rozenboim et al., 2004)* were established. When chickens during the incubation were exposed to increased temperature, the increase of the body weight *(Halevy et al., 2006b; Piestun et al., 2009)*, the percentage of *m. pectoralis superficialis* in relation to the body weight *(Piestun et al., 2009)* and the breast weight *(Collin et al., 2007)* were established.

Conclusion

Analysis of the obtained values of the observed parameters showed that modified light and temperature conditions during incubation influence on the increased proliferation of myoblasts and the increased growth of skeletal muscle cells. Such occurences are the cause of differences in carcass caracteristics between the control group and treated groups of chickens. Examination of carcass caracteristics showed that body weight, weight of drumsticks, thighs and toes in all treated groups were significantly higher than in the control group, and the weight of breasts and wings were significantly higher in the group which was incubated under modified both temperature and light conditions compared to the control group. Percentage of weight of drumsticks and thighs in relation to body weight was significantly higher in the group that was incubated under modified temperature conditions and in the group that was incubated under modified both light and temperature conditions compared to the control group.

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Uticaj modifikovanih faktora inkubacije na klanične osobine teških hibrida pilića

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Rezime

Uslovi inkubacije u velikoj meri utiču na karakteristike embrionalnog i postnatalnog razvoja pilića. Promenom faktora inkubacije teži se dobijanju što boljih proizvodnih karakteristika teških hibrida pilića. U dosadašnjim istraživanjima, utvrđeno je da primena zelene monohromatske svetlosti i termalnog

kondicioniranja tokom inkubacije imaju najpovoljniji uticaj na postnatalni razvoj pilića. U ovom radu je ispitano delovanje zelene monohromatske svetlosti i termalnog kondicioniranja tokom inkubacije na promene klaničnih osobina teških hibrida pilića. U ogledu su postavljene četiri eksperimentalne grupe. Prva grupa je inkubirana pod standardnim uslovima (37,8°C, bez osvetljenja). U drugoj grupi je u određenim vremenskim intervalima povećavana temperatura inkubacije. U trećoj grupi, inkubacija je vršena pod zelenom monohromatskom svetlosti (od 6. do 15. dana u intermitentnom režimu, a od 16. dana pod konstantnim režimom osvetljenja). U četvrtoj grupi su promenjeni i temperaturni i svetlosni uslovi inkubacije, tako da je temperatura menjana po režimu druge grupe, a svetlost po režimu treće grupe. Nakon leženja, pilići su uzgajani do 42. dana, nakon čega su određivane njihove klanične osobine. Analizom dobijenih rezultata je utvrđeno da između kontrolne grupe i svih tretiranih grupa postoje značajne razlike u telesnoj masi, masi bataka i karabataka i masi nogu, dok između prve i četvrte grupe postoje značajne razlike u masi belog mesa sa kostima i masi krila. Dobijeni rezultati se objašnjavaju uticajem promenjenih temperaturnih i svetlosnih uslova inkubacije na povećanu proliferaciju mioblasta, rast skeletnih mišićnih ćelija, kao i na aktivnost satelitskih ćelija, što se odražava i na promene u klaničnim osobinama.

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EFFECT OF PROBIOTICS ON BODY CONFORMATION OF THE FATTENING CHICKENS

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Abstract: The possibility of using different probiotic products primarily in animal food represents a special direction in the modern production of broiler meat. Intensive broiler production has been improved in the last few decades especially in the fields of genetics, nutrition and technology, in order to achieve maximum growth broilers, the optimal utilization of food and high yields, especially white meat. Considering the above mentioned research was conducted in order to examine the impact of commercial probiotic products on the body conformation of the carcasses of chickens after 42 days of fattening. In experiment was used a total of 30 chickens provenience Cobb divided into two groups (P and K). Chickens from both groups were vaccinated according to the plan and program of immunoprophylaxis, and selected technical and technological solutions of accommodation, as well as feeding and watering chickens were in accordance with the recommendations of the selectors for the floor system rearing. Chickens of experimental group (P) were given in drinking water, a commercial probiotic Probios ®, a soluble powder for poultry, product of a company Chr. Hansen A / S, Denmark. Probiotic treatment of experimental groups of chickens was conducted the first three days of life and three days during the vaccination of chickens (the day before, on the day and the day after vaccination). Measures of body conformation (the breast circumference, keel length, breast depth, drumsticks circumference, shank length and breast angle) were determined on carcasses after the fattening of 42nd day using standard tools in livestock breeding. The results obtained from this study suggest that the usage of commercial probiotics in broiler chickens in intensive fattening has proved to be statistically significant on tested measure of body conformation.

Key words: probiotic, chickens, Cobb, body conformation, fattening

Introduction

The term "probiotic" was first introduced by *Lilly and Stillwell (1965)* when describing substances which secreting some microorganisms which stimulate growth of other microorganisms while Parker gave the definition of probiotics as "organisms and substances that contribute to intestinal microbial balance" *(Parker, 1974 cited Fuller and Cole, 1989)*. Fuller later redefined the probiotics as "live microbial feed supplements which beneficially affect the animal by improving its intestinal microbial balance" *(Fuller, 1989)*. In different experimental designs were examined the various commercial and experimental probiotic preparations which contain different strains of bacteria, mold, yeast, lactose and vitamin C. Numerous authors *(Brzóska at al., 1999; Djouvinov at al., 2005; Gagić at al., 1991a, 1991b; Rešidbegović at al., 2001; Softić at al., 2003; Kavazović at al., 2004, 2009)* confirmed the positive effects of probiotic use to production parameters, body conformation and the health of the broiler chickens. Intensive broiler production has improved the last decades, especially through the segments of genetics, nutrition and technology, in order to achieve maximum growth broilers, the optimal utilization of food and high yields. The general look and feel of meatiness of chickens depends first of all from body conformation, and in this regard is the goal of producing broiler meat production, especially in the breasts, thighs and drumsticks. Considering the above mentioned our goal was to investigate the effect of commercial probiotic preparations on the body conformation of carcasses of chickens after 42 days of fattening.

Materials and Methods

In experiment was used a total of 30 chickens provenience Cobb divided into two groups (P and K). Chickens from both groups were vaccinated according to the plan and program of immunoprophylaxis, and selected technical and technological solutions of accommodation, as well as feeding and watering chickens were in accordance with the recommendations of the selectors for the floor system to hold. Chickens of experimental group (P) were given in drinking water, a commercial probiotic Probios ®, a soluble powder for poultry, product of a company Chr. Hansen A / S, Denmark. Probiotic treatment of experimental groups of chickens was conducted the first three days of life and three days during the vaccination of chickens (the day before, on the day and the day after vaccination). Measures of body conformation (the breast circumference, keel length, breast depth, drumsticks circumference, shank length and breast angle) were determined on carcasses after the fattening of 42nd day using standard tools in livestock breeding.

Results and Discussion

Absolute and relative values of measures of body conformation, and the statistical justification of the difference between the groups on chicken carcasses are shown in Table 1. Measures of conformation of broiler chickens, expressed as an index showing the ratio of body weight before slaughtering, and measure observed on the carcass, in order to minimize the effect of body mass and emphasizes the influence of the applied treatment, are also important indicators of carcass quality. Considering that the comparison of results regarding the effect of probiotics on measures of body conformation could not find the information in the available literature, our results could only compare with the results of other authors, whose experimental designs were different and other factors had an influence on these parameters.

Table 1. Measures of body conformation on chicken carcasses provenience Cobb

Groups	P (Experimental)	K (Control)
Average weight (g)	2410,12	2350,22
Breast circumference (mm)	315,67*	306,67
Drumstick circumference (mm)	163,8*	156,2
Index (g/mm)	14,71	15,05
Keel length (mm)	152,47*	144,6
Index (g/mm)	15,8	16,25
Shank length (mm)	87,53	85,73
Index (g/mm)	27,53	27,41
Breast depth (mm)	127,66	125,53
Index (g/mm)	18,88	18,72
Breast angle (degrees)	127,33***	116,4

By analyzing the results we have found the best average body weight at the end of fattening period of chickens in experimental groups. Similar to our results, the positive effects of probiotics on body weight of chickens during the experiment was confirmed by other authors *(Brzóska at al., 1999; Djouvinov at al., 2005, Ivanković et al., 1999)*. *Kavazović et al. (2009)* in their work have found positive effects of implementing a commercial probiotic Probios at the end of fattening period of 42 days on production performance (body weight, weight gain, food conversion), which is consistent with our research. The highest average value of the breast circumference of 315.67 mm was measured in experimental groups of chickens. Keel length as one measures of body conformation can be viewed from two aspects. The long keel has more space for chest muscles, and thus the curvature of the lower trunk, with one, and if given the priorities meatiness, then it

would have the advantage of relatively long keel, with the second aspect. Considering the fact, the lower index value (g/mm) indicates a more favorable conformation. Our results obtained in both cases are confirmed by a more favorable conformation in the treated chickens. Application of inactivated yeast, vitamin C and lactose in live broiler chickens of Cobb 500 strain during the fattening period of 42 days in the experimental conditions, *Softić at al. (2003)* found positive and statistically significant differences of probiotics on body weight, chest circumference and length of the keel. *Kavazović at al. (2004)* also found positive effects (increase in average body weight and weight gain, and FCR) application of the same probiotic agents in chickens for fattening than 42 days. Leg meat is, along with white breast meat, the best part of chicken carcasses. In chickens with rounded and fuller drumsticks circumference is larger, and the relative value of the expressed lower leg index indicates a more favorable conformation. By this criterion, a more favorable conformation achieved by a group of chickens treated with probiotics. Higher index of breast depth and shank length a more favorable conformation, our results are also in this view were better in the treated chickens. The highest average values breast angle we found a group chickens treated with probiotics. In comparison with the works of other authors *(Pavlovski at al. 2006; Softić, 2005)* for all parameters of body conformation, and bearing in mind, a different design of experiments and the use of other broiler strains inthem, our results obtained were higher.

Conclusion

Based on the presented results of our work and their consideration in the context of earlier studies of similar themes, we can conclude that the use of commercial probiotics Probios® proved to be justified to test a measure of body conformation in broiler chickens.

Uticaj probiotika na telesnu konformaciju pilića u tovu

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Rezime

Mogućnost korišćenja različitih probiotskih preparata pre svega u stočnoj hrani predstavlja poseban pravac u savremenoj proizvodnji brojlerskog mesa. Intenzivna brojlerska proizvodnja je poslednjih decenija unapređivana posebno u oblastima genetike, ishrane i tehnologije, s ciljem postizanja maksimalnog rasta brojlera, optimalnog iskorišćavanja hrane i visokih prinosa, posebno belog mesa. S

obzirom na navedeno izvedeno je istraživanje sa ciljem da se ispita uticaj komercijalnog probiotskog preparata na telesnu konformaciju obrađenih trupova kod pilića nakon 42 dana tova. U eksperimentu je korišćeno ukupno 30 pilića provenijence Cobb podeljenih u dve grupe (P i K). Jedinke iz obe grupe vakcinisane su prema utvrđenom planu i programu imunoprofilakse, a odabrana tehnička i tehnološka rešenja smeštaja, te ishrana i napajanje pilića, bili su u skladu sa preporukama selekcionera za podni sistem držanja. Pilići eksperimentalne grupe (P) dobijali su u vodi za piće komercijalni probiotik Probios®, topivi prašak za perad firme Chr. Hansen A/S, Danska. Probiotski tretman eksperimentalne grupe pilića bio je prva tri dana života i trodnevno pri vakcinisanju pilića (dan pre, na dan vakcinisanja i dan nakon vakcinisanja). Mere telesne konformacije (obim grudi, dužina kobilice, dubina grudi, obim bataka, dužina piska i grudni ugao) utvrđene su na trupovima nakon završetka tova 42. dana uz korišćenje standardnih pomagala u stočarstvu. Dobijeni rezultati ovog istraživanja upućuju na zaključak da upotreba komercijalnog probiotika kod brojlerskih pilića u intenzivnom tovu pokazala se statistički opravdanom na ispitivane mere telesne konformacije.

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BROILER MEAT QUALITY: THE EFFECT OF REARING SYSTEM AND LENGTH OF FATTENING PERIOD

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Abstract: Broiler meat production in recent years has been oriented towards the implementation of non-commercial rearing systems aimed at improving broiler rearing conditions, enhancing meat quality and improving the environment. This study presents productive and slaughter results of broilers reared under two non-commercial systems, including extensive indoor and free-range systems. Length of fattening period was 63 days. On days 49 and 63 of the experiment, male and female broilers were randomly selected from both rearing systems and slaughtered thereafter to be evaluated and compared for the following traits: dressing percentage and percentage yield of primal carcass cuts, as dependent upon rearing system, length of fattening period and broiler sex. The results showed a higher dressing percentage in broilers slaughtered on day 49, regardless of the higher carcass weight of broilers slaughtered on day 63. Broiler sex had a highly significant effect on the percentage yield of breast, thigh and drumstick in the dressed carcass ($P<0.01$), whereas the percentage yield of thighs was also statistically highly significantly affected by rearing system ($P<0.01$). Rearing system, length of fattening period and the interaction of these factors had no significant effect ($P>0.05$) on the percentage yield of primal cuts (breast, thigh, drumstick, wing, back and pelvis), excepting that of pelvis which was significantly affected by rearing system ($P<0.05$).

Key words: broilers, slaughter traits, rearing system, length of fattening period, sex.

Introduction

Poultry meat production in recent years has been increasingly oriented towards evaluating different rearing systems and lengths of fattening period, and their effect on meat quality. Attention has been focused on the development of new broiler rearing systems to improve rearing conditions, reduce environmental pollution and enhance meat

quality. Commercial broiler rearing systems involved cheap production of large quantities of meat over a relatively short period of time, but the system had huge disadvantages, including most notably inadequate rearing conditions – high stocking density, lack of natural light and intensive feeding, which led to serious environmental problems.

The reasons underlying the implementation of new broiler rearing systems as a replacement for the existing conventional rearing method come from legal regulations on poultry welfare. European Union countries have long since adopted directives *(VO/EWG 1538/91 and VO/EG 1804/99)* stipulating minimum broiler rearing conditions to be provided in order to satisfy non-commercial and organic poultry production standards *(Ristić, 2003)*.

A number of researchers in Serbia have made efforts to define a new broiler fattening technology through research into both newly developed genotypes *(Blagojević et al. 2009)* and new rearing methods, including extensive indoor and free-range rearing systems *(Bogosavljević-Bošković et al., 2008)* and methods involving prolongation of the fattening period *(Mitrović et al., 2004; Bogosavljević-Bošković et al. 2009, 2011)*.

This study evaluates and compares broiler meat parameters in Cobb 500 broilers designed for intensive production that were reared under two non-commercial systems i.e. under extensive indoor and free-range systems, over different lengths of the fattening period (49 and 63 days).

Materials and Methods

Cobb 500 hybrid broilers were used in the experiment. During the first 4 weeks, 200 day-old broilers were reared on deep litter floor. On day 28, the broilers were allocated to 2 groups: Experimental Group I (100 broilers) reared indoor at a stocking density of 12 birds/m^2, and Experimental Group II (100 broilers) reared both indoor at the same stocking density of 12 birds/m^2 and under free-range system at 1 m^2/bird.

Until day 42 of the fattening trial, the test broilers received two complete compound feeds containing 21% protein until day 28 and 19% protein from day 28 to day 42. Thereafter, the feed used until the end of the fattening period comprised 70% ground maize, ground barley and vitamin and mineral supplements and 30% complete feed containing 19% protein.

Length of the fattening period was 49 and 63 days.

At the end of the fattening period, 12 broilers (6 males and 6 females) were randomly selected from each length of fattening period and each rearing system for measurement of body weight and evaluation of slaughter traits during slaughter and dressing processes (according to *Rašeta and Dakić, 1984*).

The data obtained were analysed by standard variation statistics methods. Testing of the significances was performed by the following mathematical model of analysis of variance:

$$Y_{ijkl} = \mu + SG_i + DT_j + P_k + (SGDT)_{ij} + (SGP)_{ik} + (DTP)_{jk} + (SGDTP)_{ijk} + e_{ijkl}$$

i.e. according to the three-factor design 2 x 2 x 2 (2 rearing systems – RS, 2 lengths of fattening period – LF and 2 sexes – S).

The parameters tested were subjected to analysis of variance using Anova, Microsoft STATISTICA Ver. 5.0., *Stat Soft Inc. (1995).*

Results and Discussion

The data on broiler body weight before slaughter and cooled carcass weight were used to calculate the dressing percentage of broilers. Table 1 presents the dressing percentage of broilers as dependent upon different rearing systems and lengths of fattening period.

Table 1. Dressing percentage of broilers on days 49 and 63 of the fattening trial

Rearing system (1)	Length of fattening, days (2)	Sex (3)		Weight before slaughter, g	Dressed carcass	
					Weight, g	Dressing percentage , %
Non free-range	49	Male	$\bar{x}$	3023.333	2270.000	75.058
			Cv	4.810	5.791	1.668
		Female	$\bar{x}$	2502.500	1861.667	74.212
			Cv	11.617	14.048	3.459
	63	Male	$\bar{x}$	3510.000	2525.833	71.880
			Cv	16.850	18.222	3.802
		Female	$\bar{x}$	2886.667	2118.333	73.385
			Cv	3.187	3.493	1.587
Free-range	49	Male	$\bar{x}$	2771.667	2083.333	75.196
			Cv	9.117	8.831	1.650
		Female	$\bar{x}$	2641.667	1975.833	74.720
			Cv	8.305	10.016	2.275
	63	Male	$\bar{x}$	3636.667	2668.333	73.263
			Cv	12.575	14.000	1.499
		Female	$\bar{x}$	3298.333	2411.667	73.107
			Cv	4.003	5.073	2.565
F_{exp}			F_1	$42{,}24^{**}$	26,59**	7,67**
			F_2	$2{,}71^{ns}$	$1{,}49^{ns}$	$1{,}28^{ns}$
			F_3	11,44**	15,75**	$2{,}06^{ns}$
			F_{12}	$1{,}02^{ns}$	$2{,}92^{ns}$	$2{,}32^{ns}$
			F_{13}	$1{,}85^{ns}$	$0{,}25^{ns}$	$3{,}65^{ns}$
			F_{23}	$1{,}15^{ns}$	$2{,}31^{ns}$	$1{,}47^{ns}$
			F_{123}	$0{,}11^{ns}$	$0{,}25^{ns}$	$3{,}23^{ns}$

$\bar{x}$ - Average, Cv -Index of variation, ^{ns} –P>0.05; * P<0.05; ** – P<0.01

The analysis of the data given in Table 1 suggests that dressed carcass weight was expectedly highest in male and female broilers reared until day 63 under the free-

range system (2668.33gr and 2411.67gr, respectively), as body weight increased with increasing length of fattening period, resulting in the highest weight before slaughter and, hence, the highest dressed carcass weight in this group of broilers, with only the effect of rearing system and sex on dressed carcass weight being statistically highly significant (P<0.01).The dressing percentage of broilers reared until day 63 was lower than in broilers slaughtered at 49 days of age. The highest dressing percentage was obtained in broilers of both sexes reared under the free-range system until day 49, but the statistically high significance was observed only in the effect of rearing system on the trait (P<0.01), whereas the effect of the other factors tested (sex, length of fattening period, interaction) was not statistically significant (P>0.05). Mello et al. (1996), *Mitrović et al. (2004)* and *Bogosavljević-Bošković et al. (2008)* report that increasing length of fattening period results in an increase in both body weight and dressed carcass weight as well as in a decrease in dressing percentage.

Table 2. Percentage yield of primal carcass cuts of broilers on days 49 and 63 of the fattening trial

Rearing system (1)	Length of fattening, days (2)	Sex (3)		Breast	Drumsticks	Thighs/	Wings	Back	Pelvis
Non free-range	49	Male	$\bar{x}$	33,63	13,80	15,66	10,66	9,13	10,66
			Cv	2,83	3,48	5,59	3,20	8,36	10,00
		Female	$\bar{x}$	34,68	13,23	14,53	10,90	9,33	10,76
			Cv	8,67	9,08	3,47	7,28	6,71	5,73
	63	Male	$\bar{x}$	34,02	13,78	14,66	10,63	9,15	10,16
			Cv	5,50	8,92	3,18	10,46	9,04	5,85
		Female	$\bar{x}$	36,48	12,34	13,63	10,24	9,21	10,06
			Cv	4,64	4,52	3,93	5,39	8,46	4,55
Free-range	49	Male	$\bar{x}$	34,29	13,71	14,97	10,57	8,96	10,88
			Cv	6,13	4,78	2,76	3,57	3,58	5,65
		Female	$\bar{x}$	36,16	12,48	14,41	10,44	9,15	10,71
			Cv	2,66	2,23	4,52	5,39	9,41	13,79
	63	Male	$\bar{x}$	34,14	14,14	15,28	10,89	8,74	10,04
			Cv	6,83	6,77	6,06	5,68	6,45	6,14
		Female	$\bar{x}$	36,14	12,32	13,81	10,12	8,92	10,41
			Cv	4,15	2,77	3,98	6,72	7,00	9,80
F_{exp}			F_1	$0,63^{ns}$	$0,72^{ns}$	$9,18^{**}$	$1,01^{ns}$	$0,58^{ns}$	$6,07^{*}$
			F_2	$0,78^{ns}$	$0,38^{ns}$	$0,00^{ns}$	$0,29^{ns}$	$1,69^{ns}$	$0,14^{ns}$
			F_3	$13,62^{**}$	$25,96^{**}$	$27,54^{**}$	$1,45^{ns}$	$0,85^{ns}$	$0,09^{ns}$
			F_{12}	$1,47^{ns}$	$1,22^{ns}$	$3,97^{ns}$	$0,68^{ns}$	$0,25^{ns}$	$0,00^{ns}$
			F_{13}	$0,51^{ns}$	$2,18^{ns}$	$1,18^{ns}$	$2,51^{ns}$	$0,06^{ns}$	$0,10^{ns}$
			F_{23}	$0,04^{ns}$	$0,97^{ns}$	$0,04^{ns}$	$0,83^{ns}$	$0,01^{ns}$	$0,04^{ns}$
			F_{123}	$0,52^{ns}$	$0,07^{ns}$	$1,97^{ns}$	$0,00^{ns}$	$0,02^{ns}$	$0,45^{ns}$

$\bar{x}$ - Average, Cv -Index of variation, ^{ns} –P>0.05; * P<0.05; ** – P<0.01

Table 2 outlines the percentage yield of primal carcass cuts of broilers – thighs, drumsticks, breast, wings, back and pelvis.

Table 2 shows a statistically highly significant effect of broiler sex on the percentage yield of breast, thighs and drumsticks in the dressed carcass ($P<0.01$), a highly significant effect of growing system ($P<0.01$) on the percentage yield of thighs, and no statistical significance ($P>0.05$) of the effect of rearing system, length of fattening period and their interaction on the percentage yield of all primal cuts (breast, thighs, drumsticks, wings, back and pelvis), excepting that of pelvis which was significantly affected by rearing system ($P<0.05$). The analysis of the percentage yield of primal carcass cuts through the percentage yield of individual meat classes (breast, thighs, drumsticks – class I, wings – class II, back and pelvis – class III) and the effect of the factors tested (rearing system, length of fattening period and broiler sex) indicates that only rearing system had a statistically significant effect ($P<0.05$) on the percentage yield of class III meat, whereas that of class I and II meat was not significantly affected by these factors ($P>0.05$) (Table 3).

Table 3. Percentage yield of different classes of broiler meat in the dressed carcass on days 49 and 63

Rearing system (1)	Length of fattening, days (2)	Sex (3)		Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Bez ispusta	49	Male	$\bar{x}$	63,09	10,66	19,79
			Cv	1,05	3,20	4,56
		Female	$\bar{x}$	62,44	10,90	20,10
			Cv	2,58	7,28	5,18
	63	Male	$\bar{x}$	62,47	10,63	19,30
			Cv	2,22	10,46	3,45
		Female	$\bar{x}$	62,46	10,24	19,27
			Cv	2,35	5,39	5,25
Sa ispustom	49	Male	$\bar{x}$	62,97	10,57	19,84
			Cv	2,02	3,57	2,39
		Female	$\bar{x}$	63,06	10,44	19,86
			Cv	1,50	5,39	7,64
	63	Male	$\bar{x}$	63,56	10,89	18,79
			Cv	1,66	5,68	5,31
		Female	$\bar{x}$	62,26	10,12	19,34
			Cv	2,06	6,72	3,10
F_{exp}			F_1	$1,42^{ns}$	$1,01^{ns}$	$6,79^{*}$
			F_2	$0,86^{ns}$	$0,29^{ns}$	$0,31^{ns}$
			F_3	$1,10^{ns}$	$1,45^{ns}$	$0,63^{ns}$
			F_{12}	$0,01^{ns}$	$0,68^{ns}$	$0,07^{ns}$
			F_{13}	$0,37^{ns}$	$2,51^{ns}$	$0,02^{ns}$
			F_{23}	$0,08^{ns}$	$0,83^{ns}$	$0,06^{ns}$
			F_{123}	$3,29^{ns}$	$0,00^{ns}$	$0,66^{ns}$

$\bar{x}$ - Average, Cv -Index of variation, ^{ns} –P>0.05; * P<0.05; ** – P<0.01

Milošević et al. (2003), Ristić (2002), Lewis et al. (1997) and Milićević (2006) also determined that increasing length of fattening period induced an increase in major primal cuts (breast, thighs and drumsticks). *Bogosavljević-Bošković et al. (2009, 2011)* report that broilers reared without access to free range had a somewhat higher breast percentage and a lower percentage of thighs and drumsticks as compared to free range broilers. Nevertheless, the effect of rearing system and length of fattening period on the percentage of primal carcass cuts was not statistically significant ($P>0.05$), as opposed to the effect of sex on the percentage of primal carcass cuts which was found to be significant. Namely, the percentage of breast was significantly higher ($P<0.05$) in female broilers, and that of drumsticks and thighs in male broilers, as compared to broilers of opposite sex.

Conclusion

The results on the effect of rearing system and length of fattening period on broiler meat quality (dressing percentage, percentage yield of both primal carcass parts and different class meat) suggest the following:

Increasing length of fattening period induced an increase in both body weight and cooled dressed carcass weight but a simultaneous decrease in dressing percentage. The highest dressing percentage was obtained in broilers of both sexes reared under the free range system until day 49. The effect of rearing system on this trait was statistically highly significant ($P<0.01$), whereas no statistical significance ($P>0.05$) was observed in the effect of the other factors tested (sex, length of fattening period, interaction);

The highest effect on the breast and drumstick percentage of the dressed carcass was exhibited by broiler sex ($P<0.01$), whereas the thigh percentage was affected not only by broiler sex but also by rearing system ($P<0.01$). Rearing system had also a significant effect on pelvis percentage ($P<0.05$);

The proportion of class I and II meat was not affected by the treatments ($P>0.05$), whereas that of class III meat (back and pelvis) showed significant differences between indoor reared broilers and free range broilers ($P<0.5$).

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Kvalitet mesa tovnih pilića: uticaj sistema gajenja i dužine tova

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Rezime

U proizvodnji pilećeg mesa poslednjih godina teži se ka uvođenju neindustrijskih sistema gajenja u cilju poboljšanja uslova uzgoja pilića, dobijanja mesa boljeg kvaliteta i zaštiti životne sredine. U ovom radu prikazani su proizvodni i klanični rezultati tovnih pilića gajenih u dva neindustrijska sistema gajenja: ekstenzivno u živinarniku i gajenjem uz korišćenje slobodnog ispusta. Tov pilića trajao je 63 dana. Iz oba sistema gajenja, 49-og i 63-eg dana tova, metodom slučajnog uzorka odabrani su muški i ženski pilići koji su zaklani i na osnovu podataka sa linije klanja izračunati su i upoređeni: randman trupova pilića i udeo osnovnih delova trupa u zavisnosti od sistema gajenja, dužine trajanja tova i pola pilića.

Rezultati su pokazali da su veći randman klanja imali pilići zaklani 49. dana iako su pilići zaklani 63. dana imali veću masu trupa. Postojao je signifikantno visoko značajan uticaj pola pilića na udeo grudi, bataka i karabataka u obrađenom trupu ($P<0,01$), dok je na udeo karabataka u obrađenom trupu takođe imao statistički visoko značajan uticaj i sistem gajenja ($P<0,01$), a uticaji sistema gajenja, dužine trajanja tova i interakcija ovih faktora na udeo svih osnovnih delova (grudi, bataka, karabataka, krila, leđa i karlice) nisu imali statističku značajnost ($P>0,05$), izuzev na udeo karlice, gde se pokazala statistička značajnost uticaja sistema gajenja ($P<0,05$).

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GROWTH PERFORMANCES OF BROILERS HYBRO G+ PROVENIENCE

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Abstract: In this paper are presented the growth performances (live weight, average daily weight gain, feed consumption and feed conversion ratio) of broilers Hybro G+ provenience. Feeding, zoohygienic and zootechnical measures met technological normative for this provenience. Broilers were fed with the complete feed mixtures (producer „Dren“, Novi Sad) with added anticoccidial salinomycine in the amount of 66 mg/kg up to 35. day of fattening. In last week of the fattening period, salinomycine was not added (drug waiting period is 5 days). Live weight of broilers at the end of trial was 1959.74 g to 2750.48 g (average 2340.62 g). During the fattening period, average daily weight gain was 54.70 ± 5.03 g; it was in the range 45.71-64.39 g. Average daily feed consumption was 87.97 g for all fattening period and feed conversion ratio was 87.97 g.

Key words: broilers, Hybro G+ provenience, growth performances

Introduction

Chicken meat production presents 80% of total poultry meat production and has the largest increase relating to meat of other animal species. Broiler fattening period lasts short and feed conversion ratio is low. Chicken meat consumption constantly increase due to low price, attractive sensory properties and there are no cultural and religious opposites of consumption.

The goal of intensive broilers fattening is to achieve as much better growth performances as possible with fewer costs that can be observed in low daily feed consumption, low feed conversion ratio and larger daily weight gain.

Growth performances depend on genetic background (provenience), kind and quality of feed mixtures, feeding, ambient conditions and compliance of zoohygienic and zootechnical conditions of objects for animal breeding and, of course, of appearance of infective and parasitic diseases.

Genetic factor directly impact on growth performances of broiler as well as on carcass conformation *(Pavlovski et al., 2006)* and chemical composition of meat *(Ristić et al., 2007)*. One of the most important factors that impacts on growth performances of broilers is feed mixtures quality and use of some feedstuffs *(Milošević et al., 2006)*. Nowadays, there are investigations about some additives in feed mixtures for improving of performances and decreasing of some infections and intoxications. *Ivanović et al. (2009)* investigated adding of probiotics in feed mixtures, *Cabuk et al. (2004)* and *Radović et al. (2009)* adding of mineral adsorbers and *Marković et al. (2009)* adding of microelements and vitamins to feed mixtures. Growth performances also depend on, of course, of appearance of infective and parasitic diseases in broilers *(Lilić, 2007)*.

The aim of this paper was to investigate the growth performances of broilers Hybro G+ provenience during 42 days of fattening.

Materials and Methods

In this experiment one-day broiler Hybro G+ provenience was used. Farming, rearing, feeding and watering of broilers as well as use of prophylactic measures was adapted to floor raising. Before the experiment started the area for the trial was prepared. After mechanical cleaning and sanitation, equipment and floor were disinfected by biodegradable means with a wide specter of action. Litter was of wheat straw and semi-automatic drinkers, and feeders were situated. Feeding and watering were *ad libitum*. Zoohygienic and microclimatic conditions met technological normative for Hybro G+ provenience *(Technical information on Hybro G+ broilers)*.

Table 1. Feed mixtures composition, %

Index	Pre-starter	Starter	Grower	Finisher
Days	1-7	7-14	14-35	35-42
Maize	54.45	50.79	53.84	54.20
Wheat meal	2.00	2.50	1.00	4.00
Soy-bean meal	25.00	25.00	23.50	23.00
Sunflower meal	5.00	5.00	6.00	5.00
Yeast	3.00	3.00	3.00	3.00
Fish meal	5.00	4.00	3.00	-
Dehydrated alfalfa meal	-	2.00	2.00	2.00
Soy-bean oil	3.00	5.00	4.50	5.50
Dicalcium phosphate	1.00	1.20	1.30	1.10
Limestone	-	-	0.20	0.40
Salt	0.20	0.20	0.30	0.30
Lysine	0.10	0.06	0.11	0.25
Methionine	0.25	0.25	0.25	0.25
Premix	1.00	1.00	1.00	1.00

Broilers were fed with the complete feed mixtures (producer „Dren“, Novi Sad) with added anticoccidial salinomycine in the amount of 66 mg/kg to 35th day of fattening. In finisher, salinomycine was not added, because of the drug waiting period of 5 days. Feed mixture composition is presented in table 1.

Chemical composition of feed mixtures, used for broilers feeding, met broilers nutrient needs and they were in accordance with technological normative for Hybro G+ provenience. Chemical composition of feed mixtures is presented in table 2.

Table 2. Chemical composition of feed mixtures, %

Index	Pre-starter	Starter	Grower	Finisher
Days	1-7	7-14	14-35	35-42
Moisture	11.03	10.72	10.79	20.85
Ash	5.61	5.79	5.96	5.44
Crude protein	22.73	22.23	21.34	19.48
Crude fat	5.93	7.76	7.28	8.16
Crude cellulose	3.94	4.37	4.51	4.37
Non-nitrogen extract	50.76	49.12	50.12	51.69
Ca	0.95	0.97	0.99	0.81
P	0.86	0.85	0.85	0.71
ME, MJ/kg	12.92	13.23	13.12	13.43
Lysine	1.36	1.30	1.26	1.20
Methionine + cystine	0.97	0.95	0.92	0.84
Triptophane	0.31	0.31	0.29	0.27

Health of broilers was observed daily by clinical examination. During the experiment, the growth performances obtained were: live weight, daily weight gain, daily feed consumption and feed conversion ratio.

Live weight was measured on the scale with accuracy of 10^{-3} kg. Based on obtained data, average live weight and total weight gain were calculated.

During the experiment, the amount of feed mixtures was measured and according to sum of daily amounts of used feed, daily feed consumption and feed conversion ratio for each phase and for total fattening period were calculated.

Obtained results were analyzed statistically by calculating of average values, standard deviation, standard error, variation coefficient and range *(Hadživuković, 1991)*.

Results and Discussion

Live weight of broilers is presented in Table 3. Average live weight of one-day broilers was 43.12 ± 2.42 g (39.25-46.82 g). After 42 days (end of trial), live

weight of broilers was in the range of 1959.74-2750.48 g and average live weight was 2340.62 g. There was no mortality during the fattening. In accordance with the variation coefficient, which was not above 30%, live weight of broilers was statistically similar during each fattening period.

Table 3. Live weight of broilers during fattening, g, n = 25

Days	$\overline{X}$	Sx	Sd	Cv	Range
1	43.12	0.48	2.42	5.60	39.25-46.82
21	666.27	19.30	96.49	14.48	480.85-852.41
28	1189.95	37.11	177.99	14.96	912.17-1503.65
35	1808.32	45.44	192.78	10.66	1543.76-2214.25
42	2340.62	50.02	212.22	9.07	1959.74-2750.48

The average daily gain of broilers (Table 4) was 30.02 ± 4.21 g after 21 days of fattening. During the fattening, average daily gain was constantly increasing and on 28th day was 72.62 ± 14.65 g, on 35th day was 78.98 ± 10.45 g and on 42nd day was 76.04 ± 5.42 g. For the whole fattening period, average daily gain was 54.70 ± 5.03 g, ranged 45.71-64.39 g.

Table 4. Daily weight gain during the fattening, g, n = 25

Days	$\overline{X}$	Sx	Sd	Cv	Iv
21	30.02	0.84	4.21	14.03	21.04-38.39
28	72.62	4.79	14.65	20.17	52.14-96.14
35	78.98	7.23	10.45	13.24	69.21-101.51
42	76.04	6.81	5.42	7.12	59.36-85.29
1-42	54.70	4.93	5.03	9.19	45.71-64.39

Average daily feed consumption (Table 5) was increased during the each period of measuring and it was 43.19 g (1-21st day), 109.44 g (21-28th day), 150.06 g (28-35th day), 164.14 g (35-42nd day) and 87.97 g (for the whole period of fattening). Feed conversion ratio was also increased during the fattening, from 1.46 that was from 1-21st day of fattening up to 2.16 that was calculated from 35-42nd day of fattening. For whole fattening it was 1.76. In this experiment it was obtained significantly larger values for feed conversion ratio according to technological normative for Hybro G+ provenience. In this document it is stated that feed conversion ratio is in the first week 0.90, in the second 0.98, in the third 1.17, in the fourth 1.37, in the fifth 1.52 and in finishing week 1.64 *(Technical information on Hybro G+ broilers)*.

Tabela 5 Average daily feed consumption (g) and feed conversion ratio during the fattening

Indicator / Days	1-21	21-28	28-35	35-42	1-42
Average daily feed consumption	43,19	109,44	150,06	164,14	87,97
Feed conversion ratio	1,46	1,51	1,90	2,16	1,76

Live weight of broilers during and at the end of fattening is one of the main parameters that is used for assessment of growth performances and it depends on many factors such as: feed mixture quality, average daily feed consumption, feed conversion ratio and compliance of optimal zootechnical conditions on the farm and appearance of infective and non-infective diseases. Growth performances are used for estimation of success of fattening and they are one of the main parameters that impact carcass yield and broiler meat quality.

Conclusion

During the fattening, health status of broilers was well without clinical symptoms of infective and parasitic diseases.

After 42 days of fattening broilers achieved average live weight of 2340.62 g (1959.74-2750.48 g).

For whole fattening period, average daily gain was 54.70 ± 5.03 g, ranged 45.71-64.39 g.

Average daily feed consumption was increased during the each period of measuring and it was 43.19 g (1-21st day), 109.44 g (21-28th day), 150.06 g (28-35th day), 164.14 g (35-42nd day) and 87.97 g (for the whole period of fattening).

Feed conversion ratio was also increased during the fattening, from 1.46 (from 1-21st day of fattening) up to 2.16 (from 35-42nd day of fattening). For whole fattening feed conversion ratio was 1.76.

Proizvodni rezultati u tovu brojlera Hybro G+ provenijencije

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Rezime

U radu su prikazani proizvodni rezultati (masa brojlera tokom tova, prosečan dnevni prirast i utrošak hrane, konverzija hrane) brojlera Hybro G+ provenijencije. Ishrana, smeštaj, zoohigijenske i zootehničke mere odgovarale su tehnološkim normativima za ovu provenijenciju. Brojleri su hranjeni potpunim krmnim smešama (proizvođač „Dren“, Novi Sad) kojima je dodat antikokcidijal salinomicin u količini od 66 mg/kg, do 35. dana tova. U smeše korišćene za

poslednju nedelju tova, salinomicin nije dodat da bi se ispoštovala karenca leka koja za pileće meso iznosi 5 dana. Prosečna masa brojlera na kraju tova bila je od 1959,74 g do 2750,48 g (prosečno 2340,62 g). Za ceo period tova, prosečan dnevni prirast brojlera bio je 54,70 ± 5,03 g, odnosno bio je u opsegu od 45,71 do 64,39 g. Prosečan dnevni utrošak hrane bio je 87,97 g za ceo period tova, a konverzija hrane 1,76.

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THE INFLUENCE OF STRAIN AND AGE ON SOME EGG QUALITY PARAMETERS OF COMMERCIAL LAYING HENS

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Abstract: The experiment was conducted to evaluate the influence of strain and age on some egg quality parameters (egg weight and egg shell strength) of commercial laying hens. Layers of lines ISA Brown and DeKalb White were examined in period of nine months. Both lines were at same age (28 weeks) and were confined in common facility in battery cages. Laying hens were reared and fed according to standard breeding technology. Once a month 25 eggs of each line were taken to be examined for eggshell strength and egg weight, or in other words, total 450 table eggs were tested. Analises were done in Laboratory for testing egg quality by gaudges Egg Multi Tester EMT 5200 and Egg shell Gauge (Robotmation Co. Ltd., Tokyo, Japan) which have computerised equipment to examine quality and physical characteristics of eggs. Statistic analyses on results were done by computer sub-programme ANOVA and Fisher's LSD test to determine the level of statistical significant difference between examined factors. Egg weight was under significant impact of age ($P \leq 0,05$), but not under the influence of strain, although eggs of ISA Brown line were insignificantly heavier than eggs of DeKalb White line. Eggs were heaviest at layers with older age, while they were lightest at younger birds. The results have shown significant differences ($P \leq 0,05$) in eggshell strength compared to line influence (genotype) and months of age. Eggs from ISA Brown laying hens had much better and eggshell strength than those eggs from DeKalb White. Correlations between eggshell strength and egg weight were with significant ($P < 0,05$) negative value, which indicating that with increasing egg weight decreases of eggshell strength.

Key words: strain, age, egg weight, eggshell strength, layers.

Introduction

Egg quality is factor which contributes for better economy price of fertile and table eggs. Egg quality was defined by *Stadelman (1977)* as characteristics important for consumers. Economic success for a production flock is measured with total number of qualitative produced eggs *(Monira et al., 2003)*. Egg quality is presented by its weight, percentage of eggshell, thickness and strength of eggshell. Mainly, differences in eggshell quality depend of strain or line of hen *(Buss and Guyer 1982)*. Egg weight is very different between various lines of hens, and eggshell thickness is under great influence of line *(Pandey et al., 1985)*. Many researchers have raported significant genotype differences related to egg weight *(Monira et al., 2003; Anderson et al., 2004)*. Egg weight per unit area *(Arad and Mader, 1982; Izat et al., 1985)* declined with increasing age of hens, but at the same time egg size increased *(Sauter et al., 1981)*.

Genotype has direct influence on egg weight and eggshell characteristics. Many studies showed that hens with coloured feathers lay bigger eggs than hens with white feathers *(Halaj and Grofik 1994; Arent et al., 1997; Ledvinka et al., 2000; Vits et al., 2005)*. *Baumgartner et al. (2007)* determined the age impact on egg weight for hens of Leghorn strain. Eggs weight influences on weight of egg parts or components. Correlation between egg weight and weight of albumen, yolk and eggshell are high are ranged from 0.67 to 0.97 *(Zhang et al. 2005)*. *Harms et al. (1990)* established correlation ranging between egg size and eggshell in diapazon from 0.92-0.97. It is concluded that season and age, especially high summer temperatures and hens' older age have valuable influence on egg lower weight and quality (strength) of eggshell *(Nikolova and Kocevski, 2006; Nikolova et al., 2008)*.

In this study are shown the results from researches of strain and age influence on egg weight and eggshell strength at table eggs of commercial laying hens.

Materials and Methods

The research was in a farm for breeding commercial laying hens, housed in conventional battery cages, located in R.Macedonia, a country with over 300 sunny days a year and with high temperatures during summer (up to 40°C). Two lines of commercial laying hens, ISA Brown and DeKalb White were examined, both at age of 28 weeks, reared and fed according to standard breeding technology. The experiment lasted for nine months and during that period once a month 25 table eggs of each line were analysed, or total 450 eggs in order to determine egg weight and eggshell strength. The collection of eggs for analysis went on to have no major differences in weight between them in order to obtain more accurate results. Analises were done in Laboratory for testing egg quality by gaudges Egg Multi

Tester EMT 5200 and Egg shell Gauge (Robotmation Co. Ltd., Tokyo, Japan) which have computerised equipment to examine quality and physical characteristics of eggs. At the end of experiment statistic analyses on found results were done by computer program Statistica 6 and sub-programme ANOVA to determine analyses variables and Fisher's LSD test to determine existence and level of statistical significant differences between examined factors. Also, correlation between examined factors was done.

Results and Discussion

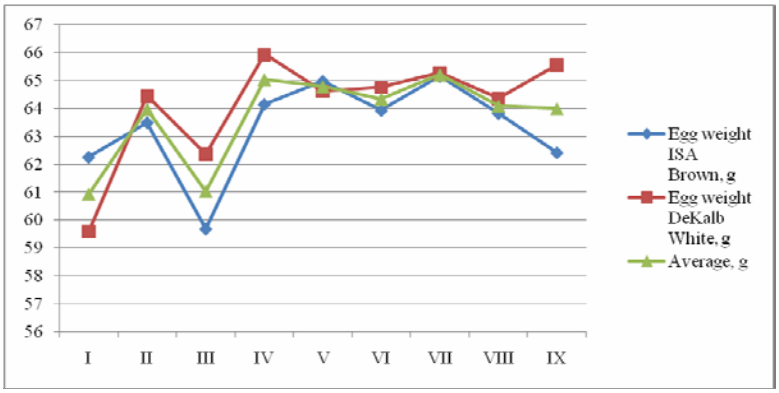
Results from egg weight measurment and eggshell strength received from two lines of commercial laying hens (ISA Brown и DeKalb White) by months are presented in Table 1. There it can be seen that weight of eggs from ISA Brown line was lowest in IIIth (59.68), and highest in VIIth (65.16) month of experiment, while for eggs of DeKalb White line had lowest value of examined parameter in Ith (59.61), and highest value in IXth (65.57) month. The average values of egg weight for laying hens from ISA Brown line were slightly bigger (64.11) compared to average values from DeKalb White line (63.72). Eggshell strength for ISA Brown line was lowest in VIth (3663.12) and highest in IXth (4050.08) compared to eggshell strength for eggs of DeKalb White line with lowest values in IVth (3227.28) and highest values in IIth (3844.96) month of investigation. From the conducted analyses of the two parameters it can be concluded that ISA Brown line gave better results.

Table 1. Egg weight (g) and egg shell strength (g/cm^2) of two different strains

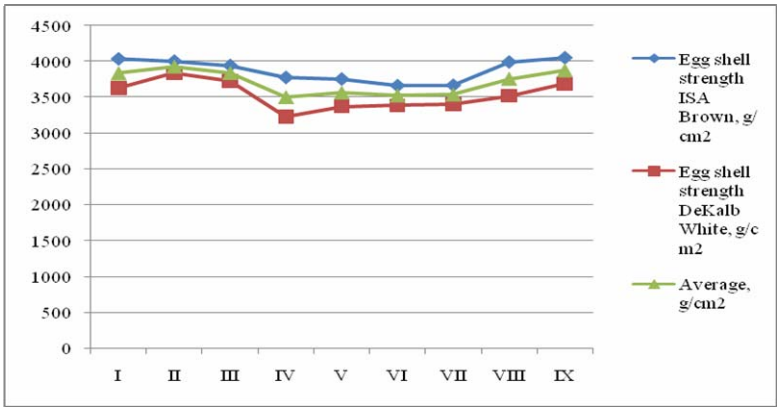
Month (age in weeks)	Egg weight ISA Brown	Egg weight DeKalb White	Average egg weight for both genotypes	Egg shell strength ISA Brown	Egg shell strength DeKalb White	Average egg shell strength for both genotypes
I (28-32)	62,26	59,61	60,93	4034,40	3627,72	3831,06
II (32-36)	63,50	64,45	63,98	4002,68	3844,96	3923,82
III (36-40)	59,68	62,38	61,03	3942,16	3728,68	3835,42
IV (40-44)	64,16	65,95	65,05	3776,16	3227,28	3501,72
V (44-48)	64,99	64,62	64,80	3755,20	3372,04	3563,62
VI (48-52)	63,94	64,77	64,35	3663,12	3385,52	3524,32
VII (52-56)	65,16	65,28	65,22	3668,92	3403,68	3536,30
VIII (56-60)	63,83	64,37	64,10	3990,52	3521,64	3756,08
IX (60-64)	62,42	65,57	64,00	4050,08	3692,00	3871,04
Average	**64,11**	**63,72**		**3875,92**	**3533,72**	

Results from Table 1 are presented in Charts (Chart 1 and 2) where differences in egg weight and eggshell quality between two commercial lines for egg production for consumation are so obvious.

In the first chart (chart1) it can be noticed that there is continually increase of egg weight with increase of poultry age for both strain during the investigation period, although for the analyses were chosen eggs with similar size.



Graph 1. Egg weight of commercial laying hens Isa Brown and DeKalb White in g



Graph 2. Egg shell strength of commercial laying hens Isa Brown and DeKalb White in g/cm^2

At the following chart (chart 2) is presented eggshell strength for both lines and their average values, where it is obvious that strength declines in older birds, something that counts for the two lines.

Analyses of variables from genotype and age influence on egg weight and eggshell strength are given in Table 2.

Table 2. Influence of strain and season on egg weight and egg shell strength

	Degr. of	Egg weight	Egg shell strength
Intercept	**1**	**0,000000***	**0,000000***
Genotype	1	0,063970	**0,000000***
Age	8	**0,000180***	**0,000210***
Month*Gen.*Age	8	0,395275	0,842386

Level of signification: * $P \leq 0.05$

The impact of age on egg weight ($P \leq 0.05$) was significant, but not for genotype (Table 2). These results are in accord with results of *Silversides and Scott (2001), Oloyo (2003), Johnston and Gous (2007), Zita et al. (2008)*, which showed that egg weight, increases with hens' age. In this research interaction among the two varibles was not significant for any of the factors, although there were differences in egg size in favour of ISA Brown strain. In previously mentioned literature more interactions between hens' genotype and age can be found. Hisex Brown and ISA Brown had a significantly ($P \leq 0.001$) higher egg weight (55.0 vs. 54.0 g) than the Moravia BSL (51.1 g) at the beginning of the experiment, but at the end of the experiment Moravia BSL produced the heaviest eggs (65.3 g).

Table 3. Inflence of genotype and age on egg weight by months (LSD-test)

	Month	Genotype	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
1	I	Isa Brown																		
2	I	DeKalb	0,11																	
3	II	Isa Brown	0,45	**0,02**																
4	II	DeKalb	0,19	**0,00**	0,57															
5	III	Isa Brown	0,12	0,97	**0,02**	**0,00**														
6	III	DeKalb	0,94	0,09	0,50	0,21	0,10													
7	IV	Isa Brown	0,25	**0,01**	0,69	0,86	**0,01**	0,29												
8	IV	DeKalb	**0,03**	**0,00**	0,14	0,37	**0,00**	**0,03**	0,28											
9	V	Isa Brown	0,25	**0,01**	0,69	0,86	**0,01**	0,29	1,00	0,28										
10	V	DeKalb	**0,03**	**0,00**	0,14	0,37	**0,00**	**0,03**	0,28	1,00	0,28									
11	VI	Isa Brown	0,31	**0,01**	0,79	0,76	**0,01**	0,35	0,89	0,22	0,89	0,22								
12	VI	DeKalb	0,13	**0,00**	0,45	0,85	**0,00**	0,15	0,71	0,48	0,71	0,48	0,62							
13	VII	Isa Brown	0,08	**0,00**	0,32	0,67	**0,00**	0,10	0,55	0,63	0,55	0,63	0,46	0,82						
14	VII	DeKalb	0,07	**0,00**	0,28	0,62	**0,00**	0,08	0,50	0,69	0,50	0,69	0,42	0,76	0,94					
15	VIII	Isa Brown	0,34	**0,01**	0,84	0,71	**0,01**	0,38	0,85	0,20	0,85	0,20	0,95	0,57	0,42	0,38				
16	VIII	DeKalb	0,20	**0,00**	0,60	0,96	**0,00**	0,23	0,90	0,34	0,90	0,34	0,79	0,81	0,63	0,58	0,75			
17	IX	Isa Brown	0,92	0,09	0,51	0,22	0,10	0,98	0,30	**0,03**	0,30	**0,03**	0,36	0,16	0,10	0,09	0,40	0,24		
18	IX	DeKalb	**0,05**	**0,00**	0,21	0,50	**0,00**	0,06	0,39	0,82	0,39	0,82	0,32	0,63	0,80	0,86	0,29	0,47	0,06	

LSD test; variable Egg weight (Spreadsheet1 in Workbook1)
Probabilities for Post Hoc Tests
Error: Between MS = 34,342, df = 432,00
($P \leq 0.05$ is **bold**)

There were important differences ($P \leq 0.05$) in eggshell strength examined under genotype and age influence and the values of the same declined for both lines of hens, though less for ISA Brown. These results are in compliance with results from *Zita et al. (2008)* where ($P \leq 0.001$) eggshell of ISA Brown eggs had greater quality during all periods of research. We came to conclusion that genotype has significant impact on eggshell quality which is not in accordance with *Basmacioglu and Ergul (2005)* results which did not confirm the same conclusion. *Yannakopoulos et al. (1994)* found no significant effect of hen age on eggshell characteristics and on eggshell thickness *(Van den Brand et al., 2004),* which is not in compliance with results from this study.

To determine the existence and level of statistically significant differences between examined factors Fisher LSD-test was used, and the results from it are presented in Tables 3 and 4. There can be noticed the combinations by age and genotypes where these significantly differences on level $P \leq 0.05$ occur.

Table 4. Inflence of genotype and age on egg shell strength by months (LSD-test)

	Month	Genotype	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
1	I	Isa Brown																		
2	I	DeKalb	**0,02**																	
3	II	Isa Brown	0,86	**0,03**																
4	II	DeKalb	0,28	0,21	0,36															
5	III	Isa Brown	0,60	0,07	0,73	0,58														
6	III	DeKalb	0,08	0,56	0,12	0,50	0,22													
7	IV	Isa Brown	0,14	0,39	0,19	0,69	0,34	0,78												
8	IV	DeKalb	**0,00**	**0,02**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**											
9	V	Isa Brown	0,11	0,46	0,16	0,61	0,28	0,88	0,90	**0,00**										
10	V	DeKalb	**0,00**	0,14	**0,00**	**0,01**	**0,00**	**0,04**	**0,02**	0,41	**0,03**									
11	VI	Isa Brown	**0,03**	0,84	**0,05**	0,30	0,11	0,71	0,52	**0,01**	0,60	0,09								
12	VI	DeKalb	**0,00**	0,16	**0,00**	**0,01**	**0,00**	**0,05**	**0,03**	0,36	**0,03**	0,94	0,11							
13	VII	Isa Brown	**0,04**	0,81	0,06	0,31	0,12	0,73	0,54	0,01	0,62	0,09	0,97	0,10						
14	VII	DeKalb	**0,00**	0,20	**0,00**	**0,01**	**0,00**	0,06	**0,03**	0,31	**0,04**	0,86	0,14	0,92	0,13					
15	VIII	Isa Brown	0,80	**0,04**	0,94	0,40	0,78	0,13	0,22	**0,00**	0,18	**0,00**	0,06	**0,00**	0,07	**0,00**				
16	VIII	DeKalb	**0,00**	0,54	**0,01**	0,06	**0,02**	0,23	0,14	0,09	0,18	0,39	0,42	0,43	0,40	0,50	**0,01**			
17	IX	Isa Brown	0,93	**0,02**	0,79	0,24	0,54	0,07	0,12	**0,00**	0,09	**0,00**	**0,03**	**0,00**	**0,03**	**0,00**	0,73	**0,00**		
18	IX	DeKalb	**0,05**	0,71	0,07	0,38	0,15	0,83	0,63	**0,01**	0,72	0,07	0,87	0,08	0,89	0,10	0,09	0,33	0,04	

LSD test; variable Egg shell strength (Spreadsheet1 in Workbook1)
Probabilities for Post Hoc Tests
Error: Between MS = 3780E2, df = 432,00
($P \leq 0.05$ is **bold**)

The correlations among age, genotype, eggshell strength and egg weight are presented in Table 4.

Table 4 Correlation between months, genotypes, eggshell strength and egg weight

	Age	Genotype	Egg shell strength	Egg weight
Age	1,000	-0,000	-0,061	0,145
Genotype	-0,000	1,000	-0,264	0,085
Egg shell strength	-0,061	-0,264	1,000	**-0,175***
Egg weight	0,145	0,085	**-0,175***	1,000

Correlations (Spreadsheet1 in Workbook1)
Marked correlations are significant at p < 0,05

Significant ($P < 0,05$) negative correlation was found between eggshell strength and egg weight (-0,175), which indicating that with increasing egg weight decreases of eggshell strength. In the table it can be noticed that correlation coefficient of eggshell strength was insignificantly negative (-0,061, -0,264, *NS*) for influence of age and genotype (-0,061, -0,264, *NS*), while correlation coefficient of egg weight with same variables was insignificantly positive (0,145, 0,085, *NS*). Discovered results are in accordance with results received from *Zita et al. (2009)* research which state high significant negative correlation between egg weight and eggshell strength (-0.100).

Conclusion

According to previously mentioned data, more important conclusions can be brought:

- Average values for egg weight and eggshell strength for the eggs of ISA Brown line were better than values for eggs of DeKalb White line.
- The influence of age on egg weight was significant ($P \leq 0,05$) but not for genotype.
- Differences in eggshell strength under genotype and age influence were significant ($P \leq 0.05$) and the same declined for both lines, though lighter for ISA Brown line
- There was significant ($P < 0,05$) negative correlation between eggshell strength and egg weight (-0,175).

Uticaj linije i uzrasti na neke parametre kvaliteta jaja komercijalnih koka nosilja

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Rezime

Ogled je izveden radi procenjivanja uticaja faktora linije i uzrasta na neke parametre kvaliteta jaja (težina jaja i tvrdoća ljuske jaja) kod komercijalnih nosilja. Bile su istestirane nosilje linije ISA Brown i DeKalb White u periodu od devet meseci. Obe linije su bile istog uzrasta (28 nedelja), smeštene u zajednički objekat u konvencionalnim baterijskim kavezima. Nosilje su odgajane i hranjene po standardnim tehnološkim normativima. Jednom mesečno skupljana su 25 jaja po liniji radi ispitivanja težine jaja i snage jajčane ljuske ili ukupno testirana su 450 jaja za konzum. Analize su izvršene u laboratoriji za ispitivanje kvaliteta jaja, a koristili su se aparati Egg Multi Tester EMT 5200 i Egg shell Gauge (Robotmation Co. Ltd., Tokyo, Japan) sa kompjuterskim računarom za ocenu kvalitativnih fizičkih osobina jaja. Statistička obrada podataka vršila se pomoću kompjuterskog podprograma ANOVA, dok je testiranje signifikantnosti rezultata izvedeno Fisherovim LSD testom. Osim toga, utvrđene su i korelacije između ispitivanih faktora.

Masa jaja je bila pod značajnim uticajem ($P \leq 0,05$) uzrasta, ali ne i pod uticajem linije (genotipa), iako su jaja linije ISA Brown bila nešto teža od onih linije DeKalb White. Jaja su imala najveću masu kod starije živine, dok su najmanju imala kod mlađih nosilja.

Rezultati su pokazali značajne razlike ($P \leq 0,05$) u snazi ljuske, u odnosu na uticaj linije i meseca (uzrasta) testiranja. Jaja nosilja ISA Brown bila su značajno tvrđa u odnosu na jaja DeKalb White nosilja. Što se tiče uzrasta, on je uticao statistički značajno kod obe linije, s tim što je jačina ljuske bila vidljivo smanjena kod starijih ptica u odnosu na ostale. Korelacije između mase jaja i jačine ljuske jaja imale su značajno negativnu vrednost ($P < 0,05$), što je potvrdilo tezu da se sa povećanjem mase jaja smanjuje jačina ljuske.

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INFLUENCE OF GENOTYPE ON EGGSHELL STRENGTH AND THE HATCHABILITY OF LAYING PARENT STOCK FLOCK

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Abstract: This research was conducted with aim to establish genotype influence on eggshell strength of fertilized eggs and its hatchability at parents stock flock of laying type. Eggs from parent's flock of lines ISA Brown and DeKalb White at age of 36-60 weeks were examined. Experiment lasted 6 months, and once a month were examined samples of 30 fertilized eggs. Results have shown significant differences ($P \leq 0.05$) in eggshell strength related to genotype influence. Eggs with brown eggshell from line ISA Brown ($P \leq 0.05$) had significantly more strength eggshell compared to eggs with white eggshells from line DeKalb White. Results from the analyses of breeding eggs hatchability, examined in the mentioned period have shown that this variable was mainly influenced by age, then by genotype. Eggs of older birds were lower hatchability compared to fertilized eggs produced of younger parent stock flock during the examination period.

Key words: genotype, eggshell strength, hatchability, parent stock flock.

Introduction

The aim of each breeder of parent stock flock is to produce more fertilized eggs with good quality, which shall grow in healthy chicks. The egg is biological system that should provide comfort surrounding to embryo while it grows into the chicks *(Narushin and Romanov, 2002)*. Each breeder is trying to provide favorable conditions and system of breeding where parents' reproductive characteristics shall enable production of standardized chicken with less costs, but with good base for their successful breeding and favorable production performances. In order to achieve this, eggshell quality has an important role, which means that it should be firm and compact, to protect the embryo from mechanical injuries and regulate gas exchange between embryo and outer environment, to protect it from bacteria and

other pathogenically organisms, to be a good source of calcium for embryo development *(Hunton, 2005)*. Eggs with thinner eggshell had 3-9 % lower hatchability than the eggs with thicker eggshell *(Bennet, 1992)*. There are many factors that have an impact on eggshell strength and its hatchability especially genotype, parents' health condition and age, season, nutrition, egg size and manipulation with it, way of breeding and conditions in incubators *(Wilson, 1997; Bucher and Miles, 2003; Coutts et al., 2006)*. Main differences in eggshell's quality depend of genotype (parents' line) and way of their breeding *(Buss and Guyer 1982).* Eggshell colour is related to eggshell quality *(Ingram et al., 2008)*. Main differences in eggshell quality are related to white and coloured hens. Brown eggs have thicker eggshell compared to white eggs *(Ledvinka et al. 2000).* It was determined that age and season as important factors, have negative influence at older birds in summer period by decrease strength of eggshell *(Nikolova and Kocevski, 2004, 2006; Nikolova et al., 2008)*. Poor eggshell quality will cause poor hatchability of fertilized eggs.

In this paper are presented the results from examination of genotype influence on eggshell strength of breeding eggs and its hatchability collected from two different parents' laying lines.

Materials and Methods

The research was in a farm for parents flock in Republic of Macedonia, country where half of the year the temperatures are very high, in specific areas permanently above 30°C degrees. Two types of parent's flocks were examined: ISA Brown and DeKalb White at age of 36-60 weeks reared and fed according to estimated breeding technology. Experiment lasted for six months and during this period of time 30 breeding eggs from each parent line were analyzed once a month in order to determine eggshell strength. The analyses were done in Laboratory for testing egg quality by gadgets Egg Multi Tester EMT 5200 and Eggshell Gauge (Robotmation Co. Ltd., Tokyo, Japan) which have computerized equipment to examine quality and physical characteristics of eggshell, yolk and egg white. In the same period, the hatchability of incubated eggs was monitored, taken from both examined parents lines.

At the end of experiment statistic analyses on found results was done by computer programmed Statistic 6 and sub-programme ANOVA to examine variable analyses and Fisher LSD test to determine the level of statistical significant difference between examined factors.

Results and Discussion

Results of eggshell strength measures which originate from two genotypes of parents lines (ISA Brown и DeKalb White) by months are presented in Table 1.

It can be seen that eggshell strength of ISA Brown eggs was thinner in I^{th}

month (3609,07), and thicker in Vth month (4105,73), while strength of DeKalb White eggshells was with lowest value for examined parameter in VIth month (3164,87), and with highest parameter value in IVth month of experiment (3753,80). Average values for eggshell strength of parent ISA Brown (3893,50) were higher than the average values for parameters of parent DeKalb White (3467,13).

Table 1. Egg shell strength of two parent stock genotypes

Egg shell strength (g/cm^2)			
Months of experiment	Age in weeks	ISA Brown	DeKalb White
I	36-40	3609.07	3419.80
II	40-44	3823.47	3309.93
III	44-48	3995.40	3708.50
IV	48-52	3870.21	3753.80
V	52-56	4105.73	3445.87
VI	56-60	3957.13	3164.87
	Average	3893.50	3467.13

The results are presented also in Graph 1 where differences in values for eggshell strength of breeding eggs from two genotypes by months can easily be seen.

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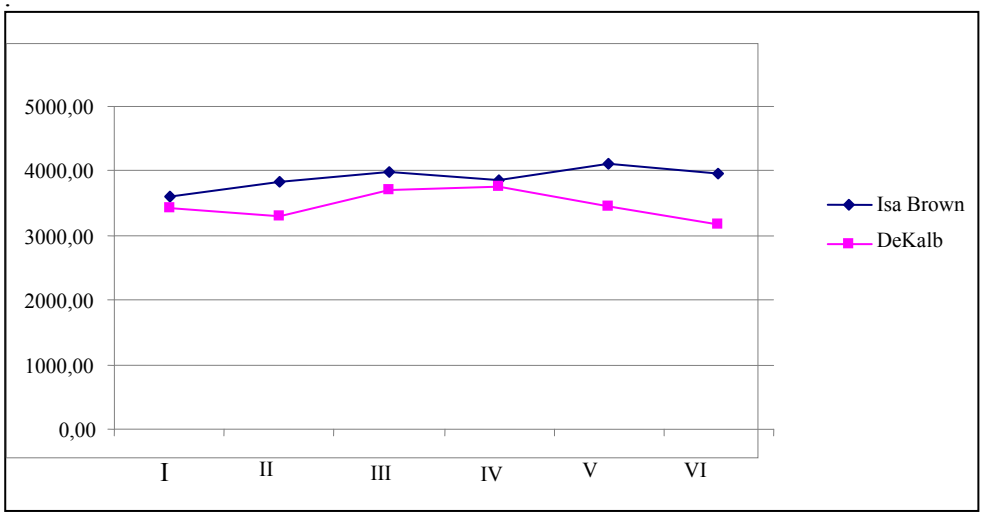


Graph 1. Egg shell strength of parent stock genotypes ISA Brown and DeKalb White in g/cm^2

The results show a condition in which the age had not significantly influence by the expected decline in the strength of the shell. Parent flock stock of

genotype ISA Brown had shown better results of eggshell strength then genotype DeKalb White in average values. Analysis of variance of the impact of the factors genotype on the variable strength of egg shell is given in Table 2.

Table 2. Influence of genotype on egg shell strength

	SS	Degr. of	MS	F	p
Intercept	2.284360E+09	1	2.284360E+09	4827.641	0.000000*
Age	3.149930E+06	5	6.299860E+05	1.331	0.253558
Genotype	7.686305E+06	1	7.686305E+06	16.244	0.000086*
Age*Genotype	2.485632E+06	5	4.971264E+05	1.051	0.390004
Error	7.570937E+07	160	4.731836E+05		

Level of signification: * $P \leq 0.05$

Generally it can be seen that genotype has statistically significant influence ($P \leq 0.05$) on eggshell strength, while age did not show any significant impact. Our results are in accordance with the certain that main differences in eggshell quality (colour, weight, strength and thickness) depend on hybrid or pure lines *(Buss and Guyer 1982).* Interaction examination between age and genotype did not give significant statistically results.

Differences between these two genotypes lead to discussions on subject how eggshell colour influences on its strength because these two are different lines, one for white eggs (DeKalb White) and the other for coloured eggs (ISA Brown). The eggs of line Isa Brown showed greater eggshell strength and it can be concluded that maybe coloured eggs have higher strength than white eggs. There are many evidences for this in literature like results of *Ledvinka et al. (2000)* according to which brown coloured eggs have thicker eggshell than white eggs, while on contrary to them *Halaj and Grofík (1994)* find out that coloured eggs have thinner eggshell. *Rayan et al. (2010)* determined the fact that eggshell strength of brown coloured eggs was higher than white coloured eggs strength, but with not statistically significant differences. On the other hand, eggshell strength depended of parents' flock age, which means that strength declines as hens grow older. In our experiment also eggshell strength declined as parents' flock was older which can be seen in the chart, especially for DeKalb White parents flock which was taken earlier to slaughtery for this reason.

For determining the existence and level of statistically significant differences between examined factors Fisher LSD-test was used and test results are presented in Table 3. There it can be seen in which combinations of months and genotypes there are some statistic differences on level $P \leq 0.05$ in eggshell strength.

Table 3. Influence of genotype and age on egg shell strength by months (LSD-test)

	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}
I month Isa Brown {1}		0.45	0.39	0.24	0.13	0.70	0.31	0.57	0.05*	0.52	0.23	0.08
I month DeKalb W {2}			0.11	0.66	0.02*	0.26	0.08	0.19	0.01*	0.92	0.06	0.31
II month Isa Brown {3}				0.04*	0.49	0.65	0.86	0.78	0.26	0.13	0.64	0.01*
II month DeKalb W {4}					0.01*	0.12	0.03*	0.08	0.00*	0.59	0.03*	0.56
III month Isa Brown {5}						0.26	0.63	0.34	0.66	0.03*	0.91	0.00*
III month DeKalb W {6}							0.53	0.86	0.12	0.31	0.39	0.03*
IV month Isa Brown {7}								0.65	0.36	0.10	0.76	0.01*
IV month DeKalb W {8}									0.16	0.22	0.48	0.02*
V month Isa Brown {9}										0.01*	0.62	0.00*
V month DeKalb W {10}											0.08	0.26
VI month Isa Brown {11}												0.01*
VI month DeKalb W {12}												

Level of signification: ***** $P \leq 0.05$

The hatchability of incubated eggs from the two genotypes in period since I to VI month is shown in Table 4. Total number of enumerated incubated eggs and total number of one-day chicken are presented, also are given monthly and general hatchability presented in percentages for both lines of parents' flocks of laying type.

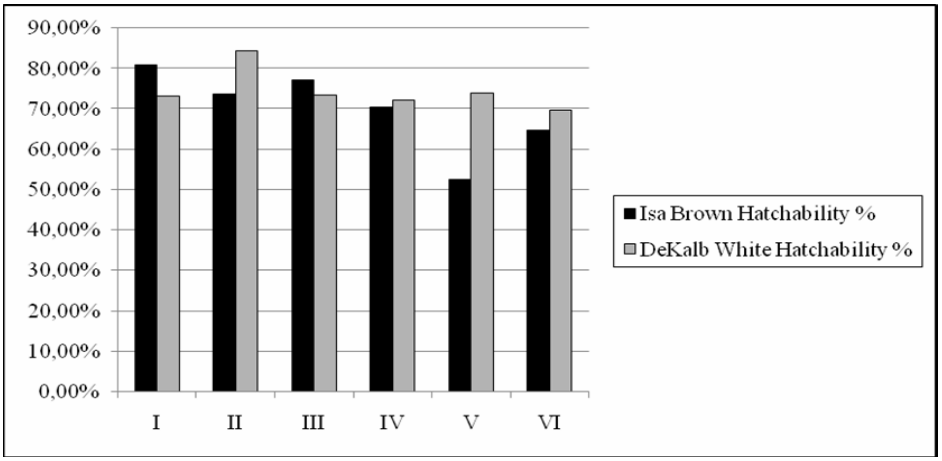
Table 4. Hatchability of fertile eggs of two parent stock genotypes by months

Months	Isa Brown			DeKalb White		
	Incubated eggs No.	Chicks hatched	Hatchability %	Incubated eggs No.	Chicks hatched	Hatchability %
I	291.690	117.878	80.82%	47.628	17.376	72.97%
II	215.964	79.499	73.62%	38.808	16.372	84.37%
III	13.608	5.250	77.16%	82.908	30.432	73.41%
IV	237.258	83.588	70.46%	81.018	29.160	71.98%
V	166.572	43.757	52.54%	107.604	39.685	73.76%
VI	130.032	41.975	64.56%	60.480	21.045	69.59%
Total	1.055.124	371.947	70.50%	418.446	154.070	73.64%

The table shows that lowest hatchability of eggshell for ISA Brown parents flock was in V^{th} (52,54%), and the highest in I^{th} (80,82%) month with average values of 70,50%. For DeKalb White line the lowest hatchability of eggshell was in VI^{h} (69,59%), and the highest in II^{th} (84,37%) month with average hatchability value of 73,64%. It can be concluded generally that the lower hatchability of chickens was in older age, while the higher was at younger birds. According to genotypes, eggs of DeKalb White line were easier for hatchability, 3,14% more in

absolute value. In this case the shell strength of incubated eggs was not in correlation with their hatchability, which needs further investigation.

In the Graph 2 are presented chart values for eggshell hatchability in relation to their genotype and months, and it are obvious the age influence and the differences between lines.



Graph 2. Hatchability of eggs from different parent stock genotypes by different age

Egg characteristics affect a lot on process of incubation and are main factor for its successfulness *(Narushin and Romanov, 2002).* Eggshell plays big part in embryo development, because it isolates the embryo from outer influences and at the same time allows gas exchange through eggshell. *Barnett et al. (2004*) found out that eggs with damaged eggshell are more exposed to bacteria and the chickens have lower weight and higher mortality, which results in lower egg hatchability (56.4% vs. 80.9%) compared to undamaged eggshell. *Bennet (1992)* compared to breeding eggs with thin and thick eggshells and came to conclusion decline of hatchability of 3 to 9% for eggs with damaged eggshell, eggs with lower humidity and contaminated with bacteria. These authors proved that eggs with thicker eggshell can be better hatchability.

Conclusion

According to previously mentioned data, more important conclusions can be brought:

- Statistically significant ($P \leq 0.05$) was genotype influence on eggshell strength, more precisely, parents from line ISA Brown produced eggs with more strength eggshell compared to parents from line DeKalb White

- Above mentioned evidence leads to conclusion that eggs with coloured eggshell (ISA Brown) had significantly ($P \leq 0.05$) more strength eggshell than the white eggs (DeKalb White).
- Age, presented by months starting from I^{th} (36-40 weeks) did not show significant influence on breeding eggshell strength although can be noticed eggshell lower quality in IV^{th} (48-52), V^{th} (52-56) and VI^{th} (56-60) months.
- Eggs hatchability was the highest in younger and the lowest in older birds. According to genotypes, eggs of DeKalb White line were easier for hatchability, 3,14% more in absolute value compared to line ISA Brown. In this case the shell strength of incubated eggs was not in correlation with their hatchability, which needs further investigation.

Uticaj genotipa na čvrstinu ljuske i lupljivosti jaja roditeljskog jata lakog tipa

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Rezime

Ovo istraživanje sprovedeno je sa ciljem utvrđivanja uticaja genotipa na čvrstinu ljuske priplodnih jaja i njihovu lupljivost kod roditeljskih parova lakog tipa. Bila su ispitivana jaja roditeljskih jata linije ISA Brown i DeKalb White na uzrastu od 36 sve do 60 nedelje. Opit je trajao šest meseci, a jedanput mesečno za ispitivanje uzimani su uzorci od po 30 oplođenih jaja.

Rezultati su pokazali značajne razlike ($P \leq 0.05$) u čvrstini ljuske u odnosu na uticaj genotipa. Jaja braon boje ljuske linije ISA Brown imali su značajno ($P \leq 0.05$) čvršću ljusku u odnosu jaja sa belom bojom ljuske linije DeKalb White. Rezultati analize lupljivosti priplodnih jaja dobijeni u ispitivanom periodu pokazali su da je ova varijabla više pod uticajem uzrasta nego genotipa. Jaja dobijena od starijih ptica imala su slabiju ljusku u odnosu na priplodna jaja proizvedena od mlađeg roditeljskog jata u toku testiranog perioda.

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THE EFFECT OF WEIGHT LOAD ON THE LEGS OF BROILERS BEHAVIOUR

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Original scientific paper

Abstract: The aim of the experiment was to study the behaviour of fast growing (FG) and slow growing (SG) broilers under the same weight load. For this purpose a harness and a suspension device were developed which alleviate a part of the weight load on the legs. The weight load in the SG birds was increased by fitting pieces of lead on the back and the breast. Using these methods the weight load of both lines was adjusted to the average weight of both lines. A total of 24 male broilers, 12 ISA S257 and 12 ROSS 308, were assigned to 4 groups of three birds each. One bird was weight adjusted, one was wearing the harness without weight adjustment and one was without harness. Duration and frequency of walking and resting behaviour were recorded. The torsional and angular deformity of tibiae were measured according to *Leterrier (1992).* The amount and thickness of the corticalis, bone density and area were measured at the middle tibia by computer tomography. The weight-reduced FG broilers showed higher activity in the 6th week of age. The weight-increased SG broilers showed less activity than the SG broilers without harness. The weight-reduced FG broilers showed similar activity as the SG broilers without harness. Total density and corticalis area were higher for the weight-reduced FG broilers than for the FG broilers without harness. The results show that weight load is the main influencing factor for differences in locomotor activity and resting behaviour of SG and FG broiler strains.

Key words: broilers, activity, resting, bone characteristics

Introduction

It is generally known, that slow growing (SG) chickens show higher locomotor activity and less resting behaviour as compared to fast growing (FG) chickens. The question whether this difference is caused by a difference in weight load or in motivation is not clear so far. The aim of the experiment was to study the behaviour of FG and SG broilers under the same weight load.

Within the last 50 years, an intensive selection took place in broilers on high growth and therefore there exists a clear reduction of the age at slaughter. Side effects of the selection of faster and higher growth are leg disorders. Up to 30% of the animals under commercial conditions have been reported to show leg disorders of different degree. In the development of leg problems, both genetic and environmental factors are involved.

Since high growth rate, low locomotor activity and leg problems appear simultaneously, it is difficult to determine the causal relationships among these traits. Fast growth can reduce the locomotor activity through the physical load. It is also assumed that, through the genetic selection for growth, the motivation of locomotor activity has been reduced. Is has further to be considered that disturbances in the development of bones and joints may cause pain and impair the locomotor activity.

Rutten et al. (2002) tried to reveal the causal relations between weight load, activity and leg disorders by reducing the weight load on legs of fast growing broilers (FB) by a special device. The weight reduced FB showed higher locomotor activity and better bone quality. However, the harness used for the reduction of weight load on the legs impaired the growth rate. Therefore it could not be clearly shown whether the increased activity was based upon the weight load reduction or on the diminished body weight. In the present study, the experiment of *Rutten et al. (2002)* was repeated with an improved device for reduction of weight load of FB. In addition, slow growing broilers (SB) were used, and were brought to the same weight load as weight reduced FB by additional load.

Materials and Methods

For this purpose a harness and a suspension device were developed which alleviate a part of the weight load on the legs. The weight load in the SG birds was increased by fitting pieces of lead on the back and the vent. Using these methods the weight load of both lines was adjusted to the average weight of both lines. The weight adjustment was made on a daily basis from 3 to 6 weeks. A total of 24 male broilers, 12 ISA S257 and 12 ROSS 308, were assigned to 4 groups of three birds each. One bird was weight adjusted, one was wearing the harness without weight adjustment and one was without harness. Observation of the broilers was performed using the "focus sampling" method in the age of six week. Duration and frequency of walking and resting were recorded and analysis was performed with the program Observer 5.0" *(NOLDUS, 2004)*. The torsional and angular deformity of tibiae and femora were measured according to *Leterrier (1992)*. The amount and thickness of the corticalis, bone density and area were measured at the proximal and distal end of tibia by computer tomography. Data was analyzed by

ANOVA using general linear models, followed by student T- test. Statistical calculations were made using program JMP, Version 5.0 (SAS Institute Inc., 2003).

Results and Discussion

Table 1. Duration of walking and resting (%), total density (TD - mg/cm3), corticalis area (CA - mm2), angular deformity of proximal and distal tibiae (TPA, TDA - °) calculated on the middle of the left tibiae in the sixth week of age

	walkin	resting	TD	CA	TPA	TDA
ISA increased weight	0,4 b	84 b	533	18	30	7
ISA with harness	1,5 a	73 a	541	22	27	4
ISA without harness	1,6 a	70 a	544	21	26	5
ROSS reduced weight	1,8 a	65 a	592	32 a	24 a	1 a
ROSS with harness	0,7 b	81 b	555	28 b	31 b	5 b
ROSS without harness	0,6 b	81 b	551	26 b	30 b	5 b

[a-b] Values within column and week with no common superscript are significantly different (P<0.05)

In the present study, the values for the locomotor activity are in the range of 0.7 to 1.6% and this is in the lower range of values reported in the literature (the time for walking of FG broilers, only 1.6% -1.8% of daylight *(Newberry and Hall, 1990)*. *Bizeray et al. (2000)* observed that in the first 3 weeks of life, walking of FG is 5%; *Bokkers (2004)* observed that from the 6th week of life, the time for the locomotor activity was only 1%). The duration of the locomotor activity of individual animals using a computer-controlled program was recorded accurately. It is therefore assumed that the reported lower levels of locomotor activity are more reliable than data from those experiments, which recorded the locomotor activity with rougher methods.

The SG broilers showed no difference in behaviour during the 6 th week of age. The weight-reduced FG broilers showed higher activity in the 6 th week of age. The weight-increased SG broilers showed less activity than the SG broilers with and without harness. The weight-reduced FG broilers showed similar activity as the SG broilers without harness.

The resting lies in areas that have been reported by other authors *(Weeks et al., 2000; Bizeray et al., 2000; Reiter and Kutritz, 2001; Bokkers, 2004)*.
Total density and corticalis area were higher for the weight-reduced FG broilers than for the FG broilers without harness. All parameters were less for the weight-increased SG broilers than for the FG broilers without harness.

These results are in compliance with results from *Rutten (2000),* who had trained animals on a treadmill, which later showed a higher cortical bone density than the untrained. Studies of fast growing broilers showed that the running training has a positive effect on the bone and bone development.
The angular deformity of tibiae was less in the weight-reduced FG broilers than at the FG broilers without harness, which coincides with results from *Reiter and Bessei (1998),* and *Rutten (2000),* where the proximal angular deformity of tibiotarsus was reduced by training in fast growing broilers.

No significant difference in angular deformity was noticed between the weight-increased SG broilers and the SG broilers without harness.

Conclusion

The results show that weight load is the main influencing factor for differences in locomotor and resting behaviour of SG and FG broiler strains.

FG broilers with reduced weight load showed more activity and better bone characteristics. SG broilers with increased weight load showed less activity but no negative influence on bone characteristic. Further experiments are necessary, to evaluate the inter relationships between locomotor activity, weight load and bone characteristics.

Efekat opterećenja nogu na ponašanje brojlerskih pilića

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Rezime

Cilj eksperimenta je bio da se prouči efekat opterećenja nogu na ponašanje brzo (FG) i sporo rastućih brojlera (SG). Za tu svrhu konstruisan je specijalan uređaj kojim je moguće olakšati brzo rastuće piliće. Težina tela kod sporo rastućih brojlera povećana je ugradnjom komada olova u specijalno napravljen ranac na leđima i grudima. Koristeći ovu metodu težina opterećenja obe linije bila je prilagođena prosečnoj težini obe linije. Ukupno 24 muška brojlera, 12 ISA S257 i 12 ROSS 308, bili su podeljeni u 4 grupe od po tri ptice. Jedan brojler je nosio ranac sa opterećenjem ili olakšanjem, drugi je nosio ranac bez težine podešavanja, a treći je bio kontrola bez ranca i opterećenja ili olakšanja. Vršeno je posmatranje pilića u šestoj nedelji života i beleženo trajanje mirovanja i kretanja pilića. Torzioni i ugaoni deformitet tibije su mereni na osnovu *Leterrier (1992)*. Debljina kortikalisa i gustina kostiju su mereni pomoću kompjuterizovane tomografije (CT). Brojleri smanjene težine tela pokazali su veću aktivnost u šestoj nedelji života.

Povećanje težine SG brojlera dovelo je do manje aktivnosti nego SG brojlera bez ranca sa opterećenjem. Ukupna gustina kosti i kortikalis oblasti su bile veće kod olakšanih FG brojlera nego kontrolnih FG brojlera. Rezultati pokazuju da je masa tela glavni faktor koji utiče na razlike u aktivnosti između SB i FB.

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THE ENVIRONMENTAL IMPACT OF POULTRY PRODUCTION

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Review paper

Abstract: The main aim of the animal production is to produce animal proteins of high value in a sustainable manner. However, sustainability is not easy to define. It is a complex phenomenon, which includes integration of economic, social, and environmental dimensions of the certain production, within a given socio-economic context. In this respect, poultry production meets first two dimensions – it provides affordable dietary item for consumers and profit for producers. It additionally generates up and downstream investment opportunities and it contributes to the development of the local economy. However, with the concentration of poultry production and increase in operation size, considerable environmental problems have occurred. During the last several decades, the environmental impact of the poultry production has received an ever-growing attention. Nowadays, producers are under heavy pressure, from different fronts, to minimize the impact of their production on the environment and to adopt welfare friendly practices. The major challenges, which will affect animal production in the future, will likely deal with the environment. In this paper, the environmental impact of poultry production and the differences in this respect between the different production systems are presented.

Key words: poultry, sustainability, environment, impact, management

Introduction

During the last several decades, sustainable development has become one of the most important developmental priorities worldwide. Although the concept of sustainable development was officially promoted in the late 1980s, there is still no precise definition of this phenomenon *(Daly, 1996; Atkinson et al., 2007).* Nevertheless, there is a consensus among the authors that each economic activity depends on the global ecosystem (for its resources and for the absorption of pollutants) and that sustainable development is possible only if an integration of

three different dimensions - economic, social and environmental is achieved *(Jovanović-Gavrilović, 2003; Rodić et al., 2007a; Boggia et al., 2010)*.

As poultry production provides an affordable dietary item of a good quality for consumers and profit for producers. Additionally it generates up and downstream investment opportunities and it contributes to the development of the local economy. Therefore, one can say that it clearly meets first two dimensions (economic and social) of sustainability. However, intensification, concentration, and an increase in operation size, have been coupled with some detrimental environmental consequences *(Rae, 1999; Wossink and Wefering, 2003; Rodić, 2006; Rodić et al., 2007a)*. Poultry is increasingly seen as a production that is not environmentally friendly and treated either as a pressure (in the "Pressure-State-Response" models), or as a driving force (in the "Driving Force-Pressure-State-Impact-Response" models) *(Willeke-Wetstein, 1998; OECD, 2003; Belini, 2005; Kostić and Rodić, 2009)*. As a result, producers are nowadays under intense pressure, from different fronts, to minimize the impact of their production on environment. According to *Tabler (2007)*, the major challenge affecting animal production in the future will be environmental.

In the paper, the environmental impact of poultry production and the differences in this respect between the different production systems are presented.

Poultry production as a polluter

Driven by economic efficiency, poultry production, like everything else in market economy, has been significantly developed and changed over the last several decades. Global poultry population has grown from 4.2 billions birds in 1961 to 17.8 billion birds in 2005 *(Hegg, 2006)*. Due to the increase in operational size, the concentration, and the intensification of poultry production, its off-site impacts have been considerably increased. Consequently, the public's perception of farmers is changing, and the public is less and less tolerant to those negative impacts *(Tabler, 2007)*. As a result, environmental regulation is increasingly developing worldwide and poultry producers have to comply with it. What always should be bear in mind is that people, not animals, are the ones who pollute, ignoring environmental constraints when managing their operations *(Rodić et al, 1999; Rodić et al., 2007b)*.

Poultry production adversely affects the environment in numerous ways – through poor management of manure and litter, waste streams from processing plants (blood, bones, feathers, etc), birds' carcasses, dust, insects, odour, etc. Furthermore, intensive poultry production is held responsible for the emission of greenhouse gasses, acidification, and eutrophication.

The environmental impact of poultry production depends on numerous factors, among which are farm size, production system, diet composition, type of bedding used, etc. It is well known that, if properly managed, waste generated in the poultry

production, especially manure and litter, could be a valuable resource, i.e. it could be used as fertilizer, soil conditioner, animal feed, or energy source *(Nahm, 2000)*. However, thanks to the large amount of waste generated (which exceed crop fertiliser requirements), content of harmful elements (such as heavy metals, pesticide residues, pathogens, pharmaceuticals, etc.), and/or unwisely management, poultry waste is often polluter instead of the valuable resource. Thus, producers have to search for environmentally sound ways of waste disposal, which inevitably affects their income.

Interest in using poultry manure and litter as a soil fertilizer for crop production has emerged in parallel with rising interest in alternative agricultural production systems, firs of all organic production. Poultry litter contains significant amounts of nutrients essential for plant growth (Table 1). Its chemical composition can vary widely depending on the type and category of poultry, type and quantity of bedding used, environmental conditions in the facility, feed source, handling of manure, etc.

Table 1. Nutrient composition of poultry litter (modified according to *Dick et al., 1998*)

	Type of manure	
	Layer	Broiler
Nutrients (%)		
Nitrogen (N)	3.3	4.1
Phosphorus (P)	2.9	2.1
Potassium (K)	3.6	2.7
Sulphur (S)	1.0	0.73
Trace elements (ppm)		
Iron (Fe)	2.040	3.254
Zinc (Zn)	403	383
Copper (Cu)	163	163

From an ecological viewpoint, organic amendments offer some advantages if compared to mineral fertilizers. In addition to nutrient supply, they improve soil structure, control erosion, and improve water-holding capacity. However, there are also some disadvantages, as unpleasant odours, high content of inorganic phosphorus, which exceeds the needs of plants, release of volatile and reactive organic compounds into the air, etc. The alternative use of poultry waste, as animal feed or as a source of energy, is rather limited due to contaminants and high moisture content. Farmers have to be aware of all these advantages and disadvantages in order to be able to find and adopt acceptable and sustainable solutions.

Environmentalists often treat poultry less environmental-friendly than other livestock productions, mainly because of the fact that birds' nutritional needs should be met exclusively by crops produced on arable land (since they cannot, unlike ruminants, digest cellulose and use less productive land). However, according to recent researches, where so called "cradle-to-grave" approach has

been applied[1] *(Williams et al., 2006; Van der Sluis, 2007; De Vries and De Boer; 2010; Van Der Werf, 2010)* poultry appears to be the most environmentally efficient of all livestock productions. In Table 2 main environmental burdens for different animal productions are given. One can see that poultry production (both meat and eggs) is more environmentally efficient than other meat productions. This is due, among other factors, very efficient feed conversation, high daily weight gain, and lower emission of enteric methane.

Table 2. Main environmental burdens of animal products *(Van der Sluis, 2007)*

Impact	Beef	Sheep Meat	Pig Meat	Poultry Meat	Eggs (20,000)	Milk (10,000 l)
	(per tonne of carcass weight)					
Primary Energy used, GJ	27	26	23	15	14	26
Global warming potential (GWP)* t CO_2 equivalent	15	17	4.9	3.6	3.8	11
Eutrophication potential (EP)** kg PO_4 equivalent	101	153	32	26	26	45
Acidification potential (AP)*** kg SO_2 equivalent	162	130	83	61	70	94

*Impacts of CO_2, N_2O, N_2O-N and CH_4 are aggregated and quantified in terms of CO_2 equivalents
**NO_3 and PO_4 leaching to water and NH_3 emissions to air are aggregated and quantified in terms of PO_4 equivalents
*** NH_3 and SO_2 (from fossil fuel combustion) emission are aggregated and quantified in terms of SO_2 equivalents

The differences in environmental impact of different poultry housing systems

Interest in alternative housing systems is growing worldwide *(Perić et al., 2007; Rodić, 2010a; Rodić 2010b)*. It is often thought that these systems are more environmental friendly. However, research done by *Williams et al. (2006)* has shown that free range and organic poultry production are more environmentally harmful than intensive production systems (Table 3).

Table 3. Comparison environmental burdens of different production systems *(Wiliams et al., 2006)*

Impact and land used	Poultry meat systems (per tonne)			Egg production system (per 20,000 eggs)		
	Traditional	Free range	Organic	Cage	Free range	Organic
Primary Energy used, GJ	12	14.5	16	13.6	15.4	16.1
Global worming potential (GWP)	4.6	5.5	6.7	5.3	6.2	7.0
Eutrophication potential (EP)	49	63	86	75	80	102
Acidification potential (AP)	173	230	264	300	312	344
Land use, ha	0.64	0.73	1.40	0.63	0.78	1.48

[1] By LCA method, all inputs into on-farm production for the observed livestock commodities (meat, milk, and eggs) were tracked back to primary resources. All activities supporting farm production (feed production processing, machinery and fertiliser manufacture, fertility building and cover crops) were also included.

While other field crops and animal products consume less primary energy and have less environmental burdens when grown organically, poultry meat and eggs are exceptions, because of the much lower bird performance *(Perić et al., 2007)* and low efficiency of feed conversion in alternative housing systems.

Conclusion

Since environmental burdens depend, to great extent, on chosen management practices, one can say that poultry industry will not threaten the environment only if both economically and environmentally acceptable management practices are applied. Unfortunately, it is still not the case and there are still many issues, related to environmental impact of poultry production, which have to be solved in the future (together with health and animal welfare issues). Unfortunately, existing scientific knowledge is sufficient for problem recognition, however it is still not capable of presenting a final solution. Farmers must be aware of ways in which their production threatens environment. Only thus, they will be able to find and adopt solutions that will provide both profitability and sustainability of poultry production.

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Uticaj živinarske proizvodnje na životnu sredinu

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Rezime

Osnovni cilj koji se postavlja pred savremenu stočarsku proizvodnju je da na održiv način proizvede visokokvalitetne proteine životinjskog porekla. Održivost, međutim, nije lako definisati. To je složen pojam koji uključuje ekonomsku, socijalnu i ekološku dimenziju određene proizvodnje, u datom društveno-ekonomskom kontekstu. U ovom smislu, savremena živinarska proizvodnja ispunjava prve dve dimenzije – ona potrošačima obezbeđuje kvalitetan prehrambeni proizvod po pristupačnoj ceni, a proizvođačima pruža mogućnost stvaranja profita, uz stvaranje mogućnosti investiranja u čitavom lancu, doprinoseći na taj način razvoju lokalne ekonomije. Međutim, sa koncentracijom i specijalizacijom živinarske proizvodnje javljaju se i sve veći ekološki problemi. U poslednje dve decenije, uticaj živinarske proizvodnje na životnu sredinu privlači sve veću

pažnju. Na proizvođače se danas, sa različitih strana, vrši sve veći pritisak da negativan uticaj na životnu sredinu svedu na minimum. Čini se da će glavni izazov pred proizvođačima u budućnosti biti upravo zadovoljavanje visokih ekoloških standarda. U radu su prikazani najznačajniji uticaji koje živinarska proizvodnja ima na životnu sredinu i razlike koje u ovom pogledu postoje između pojedinih sistema proizvodnje.

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USE EXTRUDED RAPESEED MEAL IN THE FEED OF BROILER CHICKENS

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Abstract: The aim of the study was to investigate the effects of extruded rapeseed meal in the diet on broiler performance. Chickens of Ross 398 hybrid were used in the trial. The trial lasted 42 days. Rape seed meal was extruded prior to use and included in the diet at the level of 4% (group 2) and 8% (group 3), and control group of chickens (group 1) fed the standard mixture based on corn, soybean meal and soybean grits. Chickens of the control group scored the highest ranking and realized the final body mass of 2212 g, whereas the chickens of group 2 had the final body mass of 2191 g, and chickens of group 3 with final body mass of 2148 g were the worst. Falling behind of the trial groups in relation to the control group in regard to the body mass at the end of the trial was by 0,91% group 2 and by 2,89% for group 3. Feed conversion ratio was the best in group 2, followed by control group, and the worst was the group 3 (1,882; 1,866; 1,894, respectively). The lowest mortality rate was realized by chickens of the control group, followed by group 2 and group 3 (4,00%; 4,33%; 5,33%, respectively). The control group of chickens had the best production index value, followed by group 2 and the lowest value of the production index was established for chickens of group 3 (270,95; 265,18; 255,63, respectively). It can be observed that the depression in trial groups was within the limits of toleration, which indicates that extruded rapeseed meal can be included in the diets for fattening chickens in ratio up to 10%.

Key words: extruded rapeseed meal, broilers, production traits

Introduction

Rapeseed is important plant in production of plant oils which is present in several species within *Brassica sp*. but the two most popular are B. *napus L* .and *B. rapa L.* In the last decades, numerous varieties and hybrids have been created *(Stanaćev et al,. 2002; Sovero, 1993)*. The two most widespread crops are rapeseed (Sweden) and canola (Canada), which are very similar plant species. Rapeseed has

great industrial importance because it grain contains approximately 40% of oil and 18-23% proteins *(Munoz-Valenzuela et al., 2002; Stanaćev, et al., 2007)*. Large expansion of this crop is consequence of the utilization of its oil as biodiesel fuel and raw material used in manufacturing of motor oils and hydraulic lubricants. Millions of tons of lubricants and motor oils are produced worldwide fromthis crop. By most estimates, the largest consumers of this oil will be diesel powered vehicles and lubricants used in different engines.

Important secondary products after the extrusion of oil from the grain are cake and meal, which can successfully be used in nutrition of considerable number of species and categories of domestic animals. Rapeseed meal and cakes are standardized feeds on the market and they are declared as plant protein feeds. Protein content in meal is in the range from 35-38%, contents of carbon-hydrates 15-16,5% crude fibres 11-12,5%, moisture 8-10%, ash 6-6,5%, and oil 3,7% (NRC. 1994; Canola Council of Canada, 2005). Rapeseed meal contains significant quantities of mineral matters and vitamins, especially phosphorus, of higher availability in spite of presence of phytic acid *(Keith and Bell, 1987; Stanaćev et al., 2003; Stanaćev and Kovčin, 2004; Milošević et al., 2007)*.

Disadvantage of this feed is increased content of anti-nutritional substances. Rapeseed meal contains higher quantities of glucosinolate, some varieties even over 100 μmol/g and 4-5% erucic acid *(Donald and Basin, 1990; Stanaćev et al., 2005; Milošević et al., 2010)*. The second problem is low content of metabolic energy of approx. 2000 kcal/kg *(Jokić et al., 2004; Council of Canada, 2005)*. Content of anti-nutritional substances today has been significantly reduced by creating new varieties with very small amounts of glucosinolate and erucic acid. Additional possibility to solve the issue of anti-nutritional substances is by heat treatment of feeds (expanding, toasting, extruding). There are many examples of good heat treatment where the glucosinolates and erucic acid have been reduced to minimum, which greatly reduced the depressive effect of these substances in the animal nutrition, especially in monogastric animals (pigs and poultry). Poultry fed technologically well processed/treated rapeseed meal, shpws production performance at the same level as poultry fed diets based on soybean meal or slightly lower levels *(Newkrik and Classen, 2002; Tadelle et.al., 2003; Kralik et. Al,. 2003; Stanaćev et al., 2005; Milošević et al., 2010)*.

Rapeseed meal is acceptable also to be included in diets for domestic animals because of its lower cost compared to soybean meal. According to recommendations of the Canola Council of Canada, (2005) rapeseed meal, provided that it is heat treated or made from varieties with lower content of anti-nutritional substances, can be included in diets for pigs and poultry in ratio of up to 20 %. Recommendations by *Stanaćev et al. (2005)* and *Milošević et al. (2010)*. Are that rapeseed meal can be included in diets in ratio of up to 8% without any adverse effect on production performance and health condition of fattening chickens.

Objective of this paper was to investigate the possibility of more extensive inclusion of extruded rapeseed meal in diets for fattening chickens.

Materials and Methods

Nutritional value of extruded rapeseed meal was studied on the experimental farm «Pustara» in Temerin, of the Faculty of Agriculture in Novi Sad, using Ross 308 chickens. The methodology of the trial was common. The trial was carried out on three groups of chickens, in 4 repetitions, total 300 chickens per treatment. Trial lasted 40 days. Chickens were measured once per week. Chickens were measured in group during the first, second, fourth and fifth week, and in the third and sixth week of age they were weighed individually.

Health condition of chickens was regularly controlled during the study, as well as number of dead and culled chickens recorded. Chickens were reared according to usual technology for Ross 308 hybrid. They consumed food and water ad libitum. Chemical analyses were done on samples of starter and finisher mixtures and on samples of rapeseed meal, to determine the content of crude proteins, crude fibre, crude fat and crude ash. Also, the content of following macro-elements was determined: K, Na, Ca and P.

Table 1. Composition of starter diets, %

Ingredient	Groups					
	Starter			Finisher		
	1	2	3	1	2	3
Maize	48,59	47,74	45,78	54,70	53,35	51,90
Soybean meal	29,00	26,00	23,00	23,50	20,00	15,50
Full fat soybean extruded	14,00	14,00	15,00	14,00	14,00	15,00
Rapeseed meal extruded	0,00	4,00	8,00	0,00	5,00	10,00
Yeast torula	2,50	2,50	2,50	2,00	2,00	2,00
Oil	1,50	1,50	1,50	1,50	1,50	1,50
Moncalcium Phosphate	1,30	1,20	1,20	1,30	1,20	1,20
Limestone	1,60	1,60	1,60	1,50	1,50	1,50
Salt	0,30	0,30	0,30	0,30	0,30	0,30
DL - Methionine	0,21	0,16	0,12	0,20	0,15	0,10
Premix	1,00	1,00	1,00	1,00	1,00	1,00
Total	100,00	100,00	100,00	100,00	100,00	100,00
Chemical composition						
ME MJ/kg- (calculated)	12,738	12,672	12,614	13,035	12,995	12,989
Crude protein	23,16	23,19	23,28	21,14	21,35	21,05
Fat	5,91	6,01	6,26	6,08	6,18	6,44
Crude fibre	4,21	4,42	4,64	3.99	4.22	4.42
Calcium	0,94	0,94	0,97	0,89	0,89	0,91
Phosphorus (total)	0,70	0,71	0,74	0,68	0,69	0,74
Methionine	0,57	0,53	0,50	0,53	0,49	0,48
Lysine	1,29	1,30	1,33	1,15	1,17	1,21

Group 1: Control
Group 2: Rapeseed meal extruded - content in diet 4%
Group 3: Rapeseed meal extruded - content in diet 8%

Two mixtures were used in nutrition of chickens, initial mixture – starter and final mixture – finisher. In the last trial week, chickens were fed final mixture without any coccidiostats. Composition of used mixtures is presented in Table 1.

Results and Discussion

The effect of the application of extruded rapeseed meal in fattening of chickens on production performance is presented in Table 2.

Obtained results show that introduction of extruded rapeseed meal in nutrition of fattening chickens had no significant effect on decrease of major production parameters. The data shows that final body masses were slightly lower in groups of chickens fed extruded rapeseed meal in their diets, however the difference was below 5%. The feed conversion ratio was satisfactory in all groups, but again the best value was determined in chickens of control group. The mortality ratio was usual for broiler chickens, and identical in control and trial groups of chickens which received 4% of extruded rapeseed meal in diet, and slightly higher in trial group of chickens fed diets containing 85 of this feed.

Production index which represents aggregate number (production index = average body mass (g) x % of surviving chickens x 100 / feed conversion ratio x duration of fattening, days) was the best in chickens of control group, followed by chickens of group 1, and the lowest value of production index was determined for chickens of group 2.

Obtained results are in concordance with most of literature data, although there are references where rapeseed meal even showed better production performance in chickens than soybean meal *(Munoz-Valenzuela, 2002; Kralik et al., 2003; Tadelle et al., 2003; Stanaćev et al., 2008; Milošević et al., 2010)*.

Table 2. Performance of broilers chickens (42 days)

Parameters	Groups		
	1	2	3
Mortality	12	13	16
Body weight of day-old chickens, g	42,02	42,07	42,48
Body weight, g	2212^{b}	2191^{ab}	2148^{a}
Daily weight gain, g	51,67	51,17	50,20
Mortality rate,%	4,00	4,33	5,33
Feed conversion ratio kg/kg	1,866	1,882	1,894
EPEF	271	265	256
Decrease of body weight, %	0,00	0,95	2,89

a-c, values in rows followed with same letters are significantly different at $P \leq 0,05$

Conclusion

Based on conducted investigations it can be concluded that rapeseed meal is high quality feed that can be used in nutrition of broiler chickens. It can be used as substitute for plant protein feeds in various combinations. It can be used without any fear or reservations that certain significant depression in major production parameters in broiler chickens might occur and that it will have negative consequences on their health. The recommendation to farmers is that this feed can be used in significant quantity, since inclusion of this plant feed reduces the share of other expensive plant feeds, primarily soybean meal, and this reduces the cost of complete mixtures and in general improves the profitability of production of poultry meat.

Korišćenje ekstrudirane sačme uljane repice u ishrani brojlerskih pilića

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Rezime

Cilj ispitivanja je bio efekat ekstrudirane sačme uljane repice na proizvodne parametre brojlerskih pilića. Za ispitivanje su upotrebljeni pilići Ross 308 hibrida. Ogled je trajao 42 dana. Sačma uljane repice je pre upotrebe ekstrudirana i uključena u obroke u nivou od 4% (grupa 2) i 8% (grupa 3), a kontrolna grupa (grupa 1) pilića je hranjena standardnom smešom na bazi kukuruza, sojine sačme i sojinog griza. Utvrđeno je da je kontrolna grupa pilića bila najbolja u rangu i ostvarila završnu telesnu masu 2212 g, dok je grupa 2 imala telesnu masu 2191 g, a grupa 3 je bila najlošija sa 2148 g. Zaostajanje oglednih za kontrolnom grupom u telesnoj masi na kraju ogleda je bilo ispod 0,91% grupa 2 i 2,89% grupa 3. Konverzija hrane najpovoljnija je bila grupa 2, zatim sledi kontrolna grupa i najlošija je grupa 3 (1,882; 1,866; 1,894). Najmanji mortalitet su ostvarili pilići kontrolne grupe, zatim sledi grupa 2 i potom grupa 3 (4,00%; 4,33%; 5,33%). Kontrolna grupa pilića je imala i najbolji proizvodni indeks, potom grupa 2 i najlošiji grupa 3 (270,95; 265,18; 255,63). Uočava se da je depresija u oglednim grupama bila u tolerantnim granicama što ukazuje da se ekstrudirana sačma uljane repice može uključivati u obroke za tovne piliće do 10%.

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PRODUCTION PERFORMANCE OF MEAT TYPE HYBRIDS

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Abstract: Modern broiler production is based on exploitation of meat type hybrids which are characterized by intensive growth, excellent food efficiency, strong constitution and excellent body conformation. In Serbia, many foreign proveniences are present and therefore there is a need to define a hybrid which in existing production conditions would realize the best fattening results. Objective of the study was to determine the production results of two most present line hybrids on our market, Cobb-500 and Ross-308. The following production parameters: body mass, gain, feed consumption, feed conversion, mortality and production index were monitored. One day old chickens were distributed randomly in two groups with 4 repetitions per each hybrid. In nutrition of broilers complete pellet mixtures were used, which ensured that all broiler requirements in energy, proteins, mineral substances, vitamins and essential amino acids were met. Higher final body mass was realized by Ross-308. In regard to feed conversion, mortality, feed consumption and value of production index, it is observed that Cobb-500 broilers realized better feed conversion (1,78), lower mortality (7,00%), lower consumption of feed per chicken (4238g) and higher value of production index with 316 index points compared to Ross-308 (1.82; 9,00%; 4407g; 309). Obtained production indexes for both hybrids were very high, so it can be concluded that there are no statistically significant differences between these two hybrids. It can be concluded that achieved results are satisfactory, and that hybrids have high genetic potential.

Key words: production parameters, broiler chickens

Introduction

Poultry production as one branch of livestock production is of great importance for our country since it represents major branch of economy. Poultry production enables production of large quantities of high quality products for human nutrition in very short period of time *(Ravindran and Blair, 1993)*. Within

the poultry production, intensive production of poultry meat by fattening of chickens – broilers developed and expended globally *(Rodić et al., 2003)*. In the last 30 years, intensive selection of broilers for faster growth in the shortest possible time and with the highest feed efficiency, was carried out *(Đukić Stojčić et al., 2008)*. Thirty years ago, during 56 days of rearing of fattening chickens their body mass reached only 700-800 g, whereas in the recent years, depending on the fact if fast or slow growing fattening chickens are used in fattening, they reach within 35-42 days body mass of 1,8-2,8 kg *(Grashorn and Clostermann, 2002)*. Objective of broiler rearing is production of healthy and high quality meat to meet the demands and needs of consumers *(Ljubojević et al., 2010)*. Therefore, chickens of better genetic potential, better production performance, i.e. high productive hybrids should be preferred, because they will give meat of better quality *(Stanaćev et al., 2007; Bjedov et al., 2009; Milošević et al., 2010; Petričević et al., 2011)*. So, modern poultry production today is based exclusively on utilization of line hybrids (*Rishell, 1997*). Line hybrids are divided into easy line hybrids – for production of table eggs (*Pavlovski et al., 2007*) and meat type hybrids – for production of meat *(Mitrović et al., 2010)*. All meat type hybrids are characterized by very intensive growth, good feed efficiency, strong constitution and excellent body conformation *(Arsenijević et al., 2001)*. Today many hybrids are used which are slightly different from each other. In our country the following meat type hybrids are present: Ross 308; Cobb 500; Hybro; Hubbard; Lohman*, (Bogosavljević-Bošković, 1994)* but Cobb 500 and Ross 308 are most present. Meat type hybrid Cobb 500 was created in USA. This hybrid is characterized by very fast growth rate, fast feathering, white plumage, resistance, wide and deep breasts, very muscular and rather short legs *(www.cobb-vantress.com)*. In the last decade, parent flocks were introduced in Serbia and slowly they conquered part of the domestic market. Ross 308 is also known and recognized hybrid in the world and in Serbia. It is characterized by intensive growth rate, good conversion and low mortality. It is relatively tolerant to poor housing and micro-environment conditions *(en.aviagen.com/ross-308/)*. This hybrid has been present in Serbia for some time. Objective of the present study was to determine and compare production results of two heavy line hybrids most present on our market. The following production parameters were observed: body mass, weekly gain, feed consumption, feed conversion, mortality and production index.

Materials and Methods

The trial was conducted on the experimental farm of the Department of Animal Science of the Faculty of Agriculture in Novi Sad. Two hybrids were included in the trial, Ross 308 and Cobb 500. One day old chickens of Ross 308 hybrid were delivered from AD „Topiko“, Bačka Topola, and one day old chickens

of Cobb 500 hybrid from DOO „Pileprom“, Kovilj. One day old chickens were housed in previously prepared, disinfected and heated facilities and distributed into adequate groups and repetitions. There was total of 4 repetitions per each hybrid with 75 birds per each repetition, i.e. 300 chickens per hybrid. Chickens in the facility were randomly distributed. The stocking density was 17 birds per m^2. In the broiler nutrition, from the first day to the end of fattening, complete pellet mixtures were used in order to meet all requirements and needs of broilers in regard to energy, proteins, mineral substances, vitamins and essential amino acids. During the first ten days, chickens were fed from plastic dishes, and later on from feeders. Food and water were available to chickens *ad libitum* during entire trial period. The chickens were fed three types of mixtures: starter (with 22% of crude proteins), finisher 1 (with 21% of crude proteins) and finisher 2 (19% of crude proteins). Starter mixture was used in feeding of chickens from day 1 to 10, finisher 1 mixture from day 11 to day 28, and finisher 2 to the end of the fattening, 29-42 days. Food for chickens was supplied by feed mill “Tehnooprema”. After the trial, the value of conversion for tested hybrids was calculated, and subsequently value of other production parameters. All obtained values were processed by using appropriate statistical variation methods of the statistical package STATISTIKA 9 *(StatSoft, 2010)*.

Table 1. Composition of the mixture

Feeds %	Starter	Finisher 1	Finisher 2
Corn	47,36	51,93	58,14
Wheat livestock flour	9,00	6,00	5,90
Methionine DL 99%	0,10	0,00	0,00
Soybean meal 44%	22,48	16,12	10,77
Soybean grits	16,75	22,35	21,72
Lysine L 78%	0,19	0,00	0,00
Monocalcium phosphate	1,15	1,01	0,92
Threonine 98%	0,09	0,00	0,00
Salt	0,33	0,33	0,33
Limestone	1,56	1,26	1,22
Premix	1,00	1,00	1,00
Total	100,00	100,00	100,00
Dry matter %	89,54	89,52	89,50
Crude protein	22,00	21,03	19,00
Crude fibre	5,44	6,43	6,50
Crude ash	6,25	5,62	5,20
Crude fibre	4,36	4,08	3,82
ME MJ/kg**	12,65	13,20	13,40
Lysine %	1,43	1,24	1,10
Methionine %	0,72	0,61	0,59
Methionine + cistein %	1,07	0,95	0,90
Total phosphorus	0,81	0,76	0,76
Available phosphorus	0,50	0,45	0,42

**Metabolic energy by calculation

Results and Discussion

Broiler chicken tests in the world are mainly carried out by producers of the genetic material, and results are formulated as technological norms *(Hopić et al., 1996)*. In our country, hybrids are tested even though there is no domestic breeding in poultry. Many foreign proveniences are present in Serbia and therefore there is a need to define the hybrid with best fattening results in existing production conditions. In addition to good fattening results, meat quality of tested hybrids is also important *(Vieira et al., 2004; Bianchi et al., 2006; Ristić et al., 2007)*. In consideration of all these facts, the conclusion is that it is very important to constantly repeat testings of this type. More researchers have tested production parameters of broiler chickens of various proveniences and established that certain differences in production performance of tested hybrids indicated the high genetic potential of tested hybrids *(Moran and Bilgili, 1990; Holsheimer and Veerkamp, 1992; Schutte and Pack, 1995)*. Body masses and average weekly gains of broiler chickens are presented in Table 2.

Table 2. Masses and average daily gains per weeks of fattening

Fattening, week	Cobb 500		Ross 308	
	Mass	Gain	Mass	Gain
1. day	42,2		39,3	
1	150	15,4	147	15,38
2	354	29,14	361	30,57
3	793	62,71	773	58,85
4	1212	59,85	1191	59,71
5	1917	100,71	1876	97,85
6	2542	89,28	2592	102,28

Presented results show that chickens of hybrid Ross 308 realized somewhat higher final body masses at the end of the fattening. At the beginning of the fattening, Ross 308 has slightly lower body mass compared to Cobb 500. This difference was present at the end of the first week, and in the second week, Ross 308 chickens realized higher body mass by 7g, but by the fifth week, chickens of Cobb 500 hybrid had greater masses. In the sixth week, chickens of Ross hybrid realized higher daily gains and ended the fattening with higher average mass by 50g compared to Cobb 500. By comparing the final masses, no statistically significant difference between tested hybrids was observed. Results obtained in the present study were not in concordance with results obtained by *Bogosavljević-Bošković et al. (2003)* who reported in their paper that Ross 308 had lower average body mass, poorer conversion and higher mortality compared to

Hybro G. Results pertaining to consumption of food per chicken, feed conversion, mortality and value of production index are presented in Table 3.

Table 3. Consumption of food per chicken, conversion, mortality and value of production index

Production parameter		Cobb 500	Ross 308
Food consumption per average chicken (g)		4238	4407
Conversion		1,78	1,82
Number of chickens	Start	300	300
	Finish	279	273
Died		21	27
Mortality %		7,00	9,00
Production index		316	309

In regard to other production parameters, primarily feed conversion and mortality, consumption of food and value of production index, it can be observed that chickens of hybrid realized better feed conversion, lower mortality and higher value of production index by 7 index points, compared to Ross 308 chickens. These results are in concordance with results reported by *Mandić (2006)*.

Conclusion

Looking at the results at the end of the fattening, it can be concluded that hybrids Cobb 500 and Ross 308 have high genetic potential. Higher final body mass was realized by chickens of Ross 308 hybrid. Feed conversion was somewhat better in chickens of hybrid Cobb 500, although it can be concluded that achieved results are very satisfactory and indicate extraordinary potential in utilization of food in both hybrids. Lower mortality was recorded in chickens of Cobb 500 hybrid. Obtained production indexes in both hybrids were very high, so it can be concluded that there are no statistically significant differences between these two hybrids. By analyzing the production results obtained in this test we can observe that chickens of Ross 308 hybrid realized higher final body mass, but, taking into consideration other researches, these results can not be regarded as the general statement that chickens of this hybrid were significantly superior to chickens of Cobb 500 hybrid.

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Proizvodne performanse teških linijskih hibrida

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Rezime

Savremena proizvodnja brojlera se zasniva na iskorišćavanju teških linijskih hibrida, koji se odlikuju intenzivnim porastom, odličnim iskorišćavanjem hrane, snažnom konstitucijom i odličnom konformacijom tela. Na teritoriji naše zemlje je prisutan veći broj inostranih provenijenci i postoji potreba da se definiše hibrid koji će u našim uslovima proizvodnje dati najbolje rezultate u tovu. Cilj rada je bio da se utvrde proizvodni rezultati dva najzastupljenija teška linijska hibrida na našem tržištu, Cobb-500 i Ross-308. Praćeni su sledeći proizvodni parametri: telesna masa, prirast, konzumacija hrane, konverzija, mortalitet i proizvodni indeks. Jednodnevni pilići su bili raspoređeni po principu slučajnog rasporeda u dve grupe sa 4 ponavljanja po hibridu. Ishrana brojlera je vršena kompletnim peletiranim smešama, koje su obezbeđivale zadovoljenje potreba pilića u energiji, proteinima, mineralnim materijama, vitaminima, esencijalnim aminokiselinama. Veću završnu telesnu masu ostvario je Ross-308. Kada su u pitanju konverzija, mortalitet, utrošak hrane i vrednost proizvodnog indeksa može se uočiti da je Cobb-500 ostvario bolju konverziju (1,78), niži mortalitet (7,00%), manji utrošak hrane po piletu (4238 g) i ostvario veću vrednost proizvodnog indeksa sa 316 indeksnih poena u odnosu na Ross-308 (1.82; 9,00%; 4407 g; 309). Dobijeni proizvodni indeksi kod oba hibrida su vrlo visoki, može se zaključiti da ne postoje statistički značajne razlike između ova dva hibrida. Može se konstatovati da su postignuti rezultati veoma zadovoljavajući, te da hibridi imaju visok genetski potencijal.

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CARCASS AND BREAST MEAT QUALITY OF BROILERS FEED WITH EXTRUDED CORN

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Original scientific paper

Abstract: The aim of this paper was to investigate the carcass and breast meat (nutritional, technological and sensory) quality of chickens (line ROSS 308) fed extruded corn. It was concluded that a diet with extruded corn increases chilled carcass weight and the share of breast meat in chilled carcass. Also, the breast meat of experimental group contains more protein and less free fat comparing to the control group. Based on the parameters and criteria for defining the quality of chicken breast meat (pH_u and L^*) revealed that the meat of both groups averagely corresponded to PSE quality. According to results of sensory analyzes cooked breast meat of experimental group had significantly ($P < 0.05$) preferable juiciness and tenderness.

Key words*:* feeding, extruded corn, carcass and meat quality

Introduction

Traditionally, the term 'meat quality' covers inherent properties of meat decisive for the suitability of the meat for eating, further processing and storage including retail display. Consequently, quality is now to be considered as complex and multivariate property of meat, which is influenced by multiple interacting factors including the conditions under which the meat is produced. Feeding strategy is the management factor which is most actively used as a quality control tool in the production of meat and in relation to improvement and/or control of performance, animal welfare, safety, nutritional value, and eating and technological quality *(Andersen et al., 2005)*. In the domestic production of forage mixtures, the corn has the leading position compared to other cereals, because of high energy contents (16.2 MJ/kg), starch, comparably big contents of oil and low level of cellulose *(Bekrić, 1999)*. Proper thermal process provides the reduction of thermo labile antinutrients contents, to an acceptable level, and increases digestibility of

some nutrients (protein, oil, carbohydrates), as well as the improvement of sensory features and the microbiological quality of the final product *(Kormanjoš et al., 2007)*. Parallel with the reduction of antinutrients contents it is necessary to preserve nutritionally valuable thermo labile components, so the thermal process requires a compromise between the two efforts.

New technologies used to increase the nutritional value of feed are: toasting, hydrothermal refinement, micronisation, microwave treatment, bielectrical heat treating *(Marsman et al., 1998)*. In Serbia the most often used process are extrusion and hydrothermal process *(Sakač et al., 2001; Filipović et al. 2008)*.

Determination of carcass and meat quality includes two main parts the definition of indicators for expressing the individual characteristics of quality and quantification of the inherent characteristics in relation to the overall quality. Quality rating is more complete, if it is tested and defined by larger number of properties *(Joksimović, 1977)*. Moisture, protein, fats, free amino acids and fatty acids are the most important components of meat and their qualitative and quantitative ratio determines the quality, i.e. nutritional value of meat *(Rašeta and Dakić, 1994)*. Technological meat quality traits (pH, water holding capacity, colour, cooking loss) are particularly important for industrial production and meat processing. Sensory properties: appearance, colour, texture, juiciness, aroma and taste are the most important characteristics in terms of consumers.

The aim of this study was to determine the carcass and breast meat quality (nutritional, technological and sensory) of chickens fed with extruded corn.

Materials and Methods

The experiment was carried on 3000 chickens, hybrid ROSS 308. Chickens were divided in two groups, control group (C) and experimental group (I) and fed under the same conditions in the period of 49 days. In the experimental group (I) corn was replaced with extruded corn. During whole chicken growing period water and feed were *ad libitum*. After growing and 12h starving period, 10 chickens (female) from each group were slaughtered and processed by bloodletting, scalding, plucking and evisceration and chilled. Then chickens carcasses "ready to grill" from each group were cut in the basic anatomical parts *(SFRY 1/81 and 51/88)*. Cutting and deboning of breast was applied in order to determine the breast meat yield and nutritive, technological and sensory quality of meat. Basic chemical composition of meat was estimated by determination of moisture *(JUS ISO 1442 1997)*, protein *(JUS ISO 937 1991)*, free fat *(JUS ISO 1443 1997)* and total ash *(JUS ISO 936 1998)* contents. Technological quality was evaluated by determinations of pH_u, and $colour_u$. Meat pH value was determined 24 hours post mortem (p.m.) using portable pH-meter ULTRA X. Breast meat colour was determined on the fresh cross section 24 hour p.m. using Minolta Chroma Meter CR-400, and colour characteristics were presented in u CIE *L*a*b** system

(lightness *L**, redness and greenness - *a**, yellowness and blueness - *b**) *(Robertson, 1977)*. The samples (breast meat) were cooked in the convection air oven at 175°C for 45 min. After cooking, samples were cooled at room temperature for 1 h and then analyzed for sensory characteristics. Five trained panellists, experienced in the sensory evaluation of various meat products were employed. The panellists recorded descriptive sensory evaluations. The structured linear scales were coded on a seven-point basis. Attributes included odour (1 = unpleasant, 7 = extremely pleasant), taste (1 = unpleasant, 7 = extremely pleasant), tenderness (1 = very firm, 7 = very soft), and juiciness (1 = very dry, 7 = very juicy). All data are presented as mean values. Analysis of variance (Duncan test) was used to test the hypothesis about differences among obtained results. The software package STATISTICA 8.0 *(STATISTICA 8.0, 2008)* was used for analysis.

Results and Discussion

Examination of carcass quality (Table 1) of control and experimental group shown that greater weight (1966.9 g) of cold carcass "ready to grill" was in experimental group than in control (1859.0 g), but difference was not significant ($P > 0.05$). Measured masses of chilled carcasses „ ready to grill“ were larger than the results of *Petričević et al.* (*2011*) for female chickens of Ross 308 hybrid line, what could be attributed to the prolonged fattening period of 49 days. Greater breast weight (696.6 g) and whole leg weight (569.2 g), the most important parts of the carcass, were found in chickens of experimental group. The differences between the breast and whole leg weight of control and experimental group were not significant ($P > 0.05$). Average values of back and wings weight were lower in chicken carcasses from experimental group. Again, weight of abdominal fat was lower (31.2 g) in chicken carcasses from experimental group.

Table 1. Mean values of results obtained by cutting of carcasses on main parts chickens of control and experimental groups

Group	Chilled carcass mass (g)	Breast mass (g)	Mass of whole leg (g)	Mass of back portion and tail end (g)	Mass of wings (g)	Mass of abdominal fat (g)
	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD
C	1859.0±220	657.6±93	528.2±63	412.4±44	217.6±24	35.6±6
I	1966.9±79	696.6±56	569.2±27	433.2±24	236.0±11	31.2±8
Assessment of significance						
Group	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

Chicken breast (Table 2) from experimental group had higher (P > 0.05) (540.8 g) meat weight, share of muscle in breast weight (77.5 %) and share of breast weight (27.4 %) in chilled carcass.

Table 2. Mean values of muscle breast weight (g), share (%) of muscle in breast and share (%) of breast muscle in chilled carcass of control and experimental group

Group	Muscles breast weigh (g)	Share of muscle in breast (%)	Share of breast muscle in chilled carcass (%)
	X±SD	X±SD	X±SD
C	501.8±81.7	76.1±1.9	26.9±1.3
I	540.8±60.8	77.5±3.3	27.4±2.3
Assessment of significance			
Group	P > 0.05	P > 0.05	P > 0.05

The determination of basic chemical composition of chicken breast meat (Table 3) shown lower water (73.66%) and protein (22.58%) content in breast meat from control group of chickens. Further, it can be seen from the same table that the free fat content (2.42%) was higher in breast meat of the control group comparing to experimental group (1.40%). The results are consistent with those obtained by the authors *Pavlovski and Palmin (1973), Perić et al. (1984), Džinić (1991), Džinić et al. (2009)* and *Kovačević (2001)* confirming that chicken meat contains more protein (23%) than other types of meat and less fat (1-5%), and that can be considered as dietetic foods.

Table 3. Basic chemical composition and pH value of chicken breast meat

Group	Moisture (%)	Protein (%)	Free fat (%)	Total ash (%)	pH_u
C	73.66 ± 0.1	22.58 ± 0.3	2.42 ± 0.3	1.24 ± 0.0	5.67 ± 0.1
I	74.04 ± 0.3	23.35 ± 0.3	1.40 ± 0.4	1.20 ± 0.0	5.71 ± 0.1
Assessment of significance					
Group	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

The study of technological properties of breast meat (Table 3) showed that the average pH_u value in experimental group was 5.71. Based on pH_u values, as quality parameter and based on quality criteria *(Ristić, 1981; Barbut et al., 2005)* $5.7 < pH_u < 6.1$ breast meat of experimental groups was "normal" - (red-pink, firm, dry) quality. On the other hand, based on the same criteria breast meat of control group was PSE quality. In the Table 4 it can be seen that on average breast meat of control group with lighter ($L^* = 56.33$), comparing to experimental group ($L^* = 53.91$) but this difference was not significant (P > 0.05). Based on the brightness parameter *(L*)* and criteria *(Qiao et al., 2001; Lara et al., 2003)* for PSE chicken

meat quality ($L^* > 52$) meat from control and experimental groups were, on average, PSE quality. Furthermore, the average share of red colour *(a*)* in chicken breast meat of control group was significantly ($P < 0.05$) higher than in experimental group.

Table 4. Colour and sensory characteristics of chicken breast meat

Group	Colour characteristics			Sensory characteristics			
	*L**	*a**	*b**	Odour	Taste	Juiciness	Tenderness
C	56.37 ± 3.0	3.37[a] ± 0.9	7.27 ± 1.6	6.50 ± 0.0	6.25 ± 0.0	4.71[b] ± 0.1	5.71[b] ± 0.1
I	53.91 ± 3.3	1.92[b] ± 0.8	6.88 ± 2.5	6.42 ± 0.1	5.96 ± 0.2	4.96[a] ± 0.1	5.88[a] ± 0.0
Assessment of significance							
Group	P > 0.05	P < 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P < 0.05

[a,b]- in the same column difference significant ($P < 0.05$);

Result of sensory evaluation of cooked breast meat samples of control and experimental group are presented in the Table 4. The odour and taste of chicken cooked breast meat from control (C) and experimental group (I) were averagely evaluated as "very good" (Table 4). On the other hand, juiciness and tenderness were averagely evaluated as "good" and "very good" and samples of experimental group had significantly ($P < 0.05$) better marks for these attributes.

Conclusion

The diet with extruded corn resulted in increased weight of chilled carcass, higher share of breast meat in chilled carcass, higher protein and reduced free fat content in breast meat ($P > 0.05$), comparing to control group. Additionally, it was found that the technological quality of breast meat of the control and experimental groups, according to parameters and criteria for quality estimation (pH_u, L^*), averagely corresponds to PSE meat quality. According to results of sensory analyzes cooked breast meat of experimental group had significantly ($P < 0.05$) preferable juiciness and tenderness.

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Kvalitet trupa i mesa grudi brojlera hranjenih ekstrudiranim kukuruzom

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Rezime

U ovom radu je ispitan kvalitet trupa i nutritivni, tehnološki i senzorni, kvalitet mesa grudi pilića hranjenih ekstrudiranim kukuruzom. Ispitivanja su obavljena na pilićima hibridne linije Ross 308. Zaključeno je da ishrana sa ekstrudiranim kukuruzom utiče na povećanje mase ohlađenog trupa i veći udeo mesa grudi u ohlađenom trupu ($P > 0.05$). Takođe, meso grudi eksperimentalne grupe sadrži više proteina i manje slobodne masti u odnosu na ($P > 0.05$) meso kontrolne grupe. Na osnovu parametara i kriterijuma za definisanje kvaliteta mesa grudi pilića (pH_k i L^*) utvrđeno je da je meso obe ispitane grupe prosečno BMV kvaliteta. Rezultati senzorne analize ukazuju da je toplotno obrađeno meso eksperimentalne grupe značajno bolje ($P < 0.05$) sočnosti i nežnosti.

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WELFARE ASSESSMENT OF THREE CHICKEN BREEDS (*Gallus gallus domesticus)* UNDER DIFFERENT PRODUCTION SYSTEMS

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Original scientific paper

Abstract: The objective assessment of welfare in poultry under different rearing systems has gained an increasing importance. In particular, organic rearing systems are examined in relation to improving poultry welfare and the quality of poultry production at a worldwide scale. The aim of the present study was to assess the welfare of parent flocks – *White Plymouth Rock*, *Barred Plymouth Rock* (*Line F*) and *New Hampshire*, reared under two production systems – indoor on a litter and organic (sleep houses and walk yards). The rearing and microclimatic conditions, the poultry behaviour and blood plasma corticosterone concentrations under both farming systems were examined. The welfare assessment score of *White Plymouth Rock*, *New Hampshire* and *Barred Plymouth Rock*, reared indoor on a litter was 60%, whereas in an organic production system – 90%. The higher welfare of birds reared organically was determined by the greater number of birds spending their time in dust bathing ($P<0.01$) and feather cleaning ($P<0.05$) as well as by fewer episodes of aggression ($P<0.01$) and plasma corticosterone levels ($P<0.01$), as compared to chickens reared indoor on a litter.

Key words: welfare assessment, rearing, chicken behaviour, corticosterone

Introduction

The objective assessment of welfare in poultry under different rearing systems has gained an increasing importance. The organic rearing systems are examined in relation to improving poultry welfare and the poultry production quality at a worldwide scale *(Anonymous, 2006 a,b).*

Stress has a negative impact on poultry welfare, and the stress itself could be provoked by a number of factors: technology drawbacks, poor microclimatic rearing conditions – increased ambient temperature and humidity, permanent exposure to ammonia concentrations close to maximum allowed, inappropriate

light regimen etc. *(Smith and Teeter, 1981; Hocking et al., 2001; Sahin et al., 2002; De Jong et al., 2002a,b*; *Sahin and Kucuk, 2003).*

Poultry behaviour is the most reliable indicator of welfare (*Sherwin and Kelland, 1998; Broom, 2001;Platz at al., 2003; Martrenchar et al., 2001; Dawkins, 2003; Linares and Martin, 2010)*. Another sensitive parameter of stress-induced poor welfare in birds is blood plasma corticosterone (*Sahin et al., 2002; Gonzales-Esquerra and Leeson*, 2006; *Lin et al., 2006; Mormède et al., 2007)*.

At a worldwide scale, there is an increasing interest in objective, mathematical evaluation of poultry welfare with regard to the unbiased comparison of physiological comfort of birds under different production systems *(Huber-Eicher and Wechsler, 1997; Dawkins, 2003; De Mol et al., 2006; Linares and Martin, 2010)*.

The aim of the present research was to assess the welfare of three breeds of broiler breeders reared under two production systems – indoor on a litter in boxes and organic, in sleep houses and walk yards. For this purpose, we have evaluated microclimatic parameters, their behaviour and blood plasma corticosterone concentrations.

Materials and Methods

The experiments were carried out with 312 broiler breeders (*Gallus Gallus domesticus)* from three breeds – *White and Barred Plymouth Rock* (*Line F*) and *New Hampshire*, 45 weeks of age, reared under two production systems – indoor on litter and organic (sleep houses and walk yards).

The investigations were carried simultaneously during the second half of May at two locations: the Poultry Production Base at the Institute of Animal Sciences – Kostinbrod and at the Poultry farm of the Department of Animal Science, Agriculture University – Plovdiv. The sex ratio in the flocks was 1 rooster per 8 hens.

In the first production system, broiler breeders were reared in groups of 52 birds from each strain on litter in group boxes with a density of 4 hens/m^2, in one premise with natural ventilation. They were fed by round tubular feeders ensuring feeding and drinking widths of 4 and 3 cm respectively, as required by zoo hygienic norms (*Anonymous, 2006a)*. For egg-laying, single-floor wooden nests with dimensions 30/30/40 cm were provided (4 hens in one nest).

In the organic production system, 52 hens from each breed were placed in heat-insulated sleep- houses (size 3.50/2.50/2.75 m) and walking yards (9.20/24 m). The houses included 3 perches, 2 m in length, and 8 two-floor wooden nests of 30/30/40 cm each. The light intensity was 1:10. In the bottom of the southern wall of houses, there was a 30/40 cm rectangular opening for access to the yard. In the middle of each yard, there were perennial broadleaf trees. Yards were provided with two rows of tubular feeders and with watering troughs ensuring

feeding and drinking widths of 10 and 3 cm, respectively as required by zoohygienic norms (*Anonymous, 2006a)*. The chickens were fed ad libitum with a standard ration according to their category.

Microclimatic conditions were determined by routine methods. The temperature and the relative humidity of air were measured by a with a weekly thermohygrograph; the velocity of the air motion – with a catathermometer. the light intensity – with a luxmeter. the concentration of ammonia – with indicator tubes and Drager ammonia sensor and calculated in ppm.

Blood samples for analysis were obtained from six birds from each group on May 19 from v. subcutanea ulnaris in sterile vacutainers. The duration of each manipulation was not longer than 2 min. i.e. lower than the maximum time that did not influence plasma corticosterone concentrations in birds *(Lagadic et al., 1990)*.

The behaviour of parent flocks was recorded with a video camera for 12 hours during 4 consecutive days – from May 15 ^{to} 18. Based on the recordings we prepared ethogrammes as per Wojcik & Filus. 1997. During the ethological study we counted the number of birds engaged in specific forms of behaviour: ingestive (ingestion of water or food). gregarious (moving. lying. egg-laying. dust bathing and feather cleaning) and agonistic and sexual behaviour as per *Popova-Ralcheva (1994)*.

Plasma corticosterone levels were assayed with immunoenzymatic ELISA kit (Corticosterone ELISA RE52211, IBL Gesellschaft fur Immunchemie und Immunbiologie MBH, Hamburg, Germany) in the Laboratory of Innate Resistance Investigation at the Veterinary Genetics and Breeding Unit, Department of General Animal Breeding, Faculty of Veterinary Medicine – Stara Zagora.

The welfare assessment score was calculated by the system of *Bozakova (2004)*. It is based on the scientific concept of animal welfare of the UK Farm Animal Welfare Council *(FAWC, 1995)*. It included the so-called five freedoms guaranteeing poultry welfare which are given a specific score: 2, 1 and 0, depending on expression of a particular behaviour and plasma corticosterone concentrations under different production systems.

The utmost importance in welfare assessment was attributed to the lack of pain, injury and disease. When some of these were present, regardless of other signs, the score was zero. The final score was obtained as a sum of numerical expressions of all freedoms and compared to the maximum possible score of 10, expressed in percentage.

The statistical processing of the results was performed by means of one-way ANOVA using the GraphPad InStat 3.06 software at level of significance $P<0.05$.

Results

The data for the microclimatic parameters of both production systems are given in Table 1.

Comparing the average temperature, air humidity and air movement velocity in the birds' living area with the veterinary requirements for animal breeding facilities, *Anonymous (2006a)* we determined that they were within the norms with some exceptions.

In the indoor production system, air humidity and ammonia concentration were at the upper allowed limits. For the organic system, the higher light intensity was due to the open-air rearing to which birds have adapted.

There were significant differences in the gregarious (lying, feather cleaning, dust bathing) and agonistic behaviour (aggression) behaviour of the flocks reared under different production systems (table 2). The number of lying *New Hampshire* and *Barred Plymouth Rock* hens was higher ($P<0.05$) as did dust bathing birds from all breeds reared organically compared to those reared indoor ($P<0.01$). The birds exhibiting aggression were also fewer ($P<0.01$).

Table 1. Microclimate parameters for the broiler breeder flocks at the different rearing systems

Rearing system	Temperature, (^{0}C)	Humidity, %	Ventilation, m/s	NH_3, ppm	Lux, Lx
Indoor on litter - 4 hens/m^2	18.5±0.2	70.13±0.50	0.27±0.005	12.54±0.36	31.25±1.74
Organic system-sleep houses and walk yards	20.5±2.5	61.88±0.54	0.50±0.04	follow	74.50±15.50
Reference values*	18 – 25	50 – 70	0.2 – 0.5	< 15	30 – 60

*Reference values as per *Anonymous (2006 a)*

Table 2. Number of White Plymouth Rock, New Hampshire, and Barred Plymouth Rock birds exhibiting a specific type of behaviour under two production systems (mean ±SEM, n=52).

	White Plymouth Rock		New Hampshire		Barred Plymouth Rock	
Behaviour	Indoor on litter	Organic system	Indoor on litter	Organic system	Indoor on litter	Organic system
Feeding	12.82±1.44	13.50±1.22	12.90±1.42	13.82±1.26	13.00±1.38	13.64±1.28
Drinking	8.54±0.44	8.36±0.62	8.50±0.46	8.36±0.62	8.60±0.48	8.36±0.62
Egg-laying	3.22±0.50	2.86±0.46	3.18±0.50	2.86±0.46	3.22±0.50	2.86±0.46
Moving	13.90±0.62	14.86±0.90	13.72±0.62	14.72±0.92	14.00±0.62	14.60±0.84
Lying	3.36±0.52	2.28±0.42	3.54±0.54$^{\#}$	2.28±0.42$^{\#}$	3.54±0.54"	2.28±0.42"
Feather cleaning	0.82±0.14	1.08±0.26	0.78±0.14$^{\#}$	1.28±0.26$^{\#}$	0.86±0.16	1.10±0.26
Dust bathing	0.64±0.16**	1.82±0.26**	0.86±0.18$^{\#\#}$	2.00±0.24$^{\#\#}$	1.00±0.20""	2.10±0.24""
Aggression	4.40±0.24**	0.64±0.18**	4.18±0.24$^{\#\#}$	0.64±0.18$^{\#\#}$	3.96±0.24""	0.50±0.12""
Sexual behaviour	4.50±0.40	4.50±0.40	4.50±0.40	4.50±0.40	4.50±0.40	4.50±0.40

***P<0.01 between White Plymouth Rock groups; $^{\#}$P<0.05; $^{\#\#}$P<0.01 between New Hampshire groups;*

" P<0.05; ""P<0.01 between Barred Plymouth Rock groups.

Changes in plasma corticosterone concentrations in breeder flocks (Fig. 1) showed statistically significantly higher levels for the three breeds when reared indoor on a litter (P<0.01).

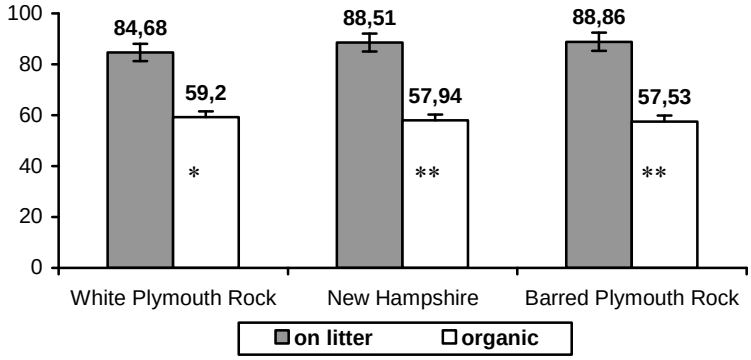

Figure 1. Plasma corticosterone (nmol/L) levels in *White Plymouth Rock*, *New Hampshire* and *Barred Plymouth Rock* breeders reared under two different production systems (mean ±SEM, n=6). *P<0.05; **P<0.01 between two production systems within each breed.

Table 3 presents the total scores of welfare assessment for the two production systems studied. In organically reared birds, the freedom from discomfort, freedom from fear and stress were given a bigger score (2) due to the more intensive dust bathing (P<0.01), lower aggression (P<0.01) and plasma corticosterone (P<0.05, P<0.01) as compared to birds reared indoor.

Table 3. Welfare assessment scoring system under two different production systems

Welfare parameters	*White Plymouth Rock*		*New Hampshire*		*Barred Plymouth Rock*	
	Indoor on litter	Organic system	Indoor on litter	Organic system	Indoor on litter	Organic system
1. Freedom from thirst and hunger	2	2	2	2	2	2
2. Freedom from discomfort	1	2	1	2	1	2
3. Freedom from pain, injury and disease	1	1	1	1	1	1
4. Freedom to express normal behaviour	1	2	1	2	1	2
5. Freedom from fear and distress	1	2	1	2	1	2
Total score	6	9	6	9	6	9
Welfare assessment score, %	0.6 60	0.9 90	0.6 60	0.9 90	0.6 60	0.9 90

Discussion

The rearing of many birds indoor on litter, air humidity and air ammonia concentration were close to upper allowed limits and thus, act as stressors for birds, stimulating the secretion of corticosterone. This reflects upon the

behaviour of all three studied breeds - *White Plymouth Rock, New Hampshire, Barred Plymouth Rock*. When reared indoor, the number of birds performing feather cleaning and dust bath was significantly lower ($P<0.01$) and their aggressiveness increased ($P<0.01$), as compared to same breeds reared organically.

This confirmed the higher welfare of the experimental birds. Similar were the results of *Iliev et al. (1999)* which claimed that dust bathing was the uppermost demonstration of comfort in birds. According to *Sherwin and Kelland (1998)* and *Stoyanchev et al. (2006)* dust bathing is a reliable indicator of poultry welfare. That is why the freedom from discomfort and the freedom to express the normal behaviour organically reared breeders were assessed with a higher score (2).

Breeders reared indoor exhibited higher level of aggressions ($P<0.01$) compared to those reared organically. This is parameter of worsened welfare. Similar results were reported by *Popova-Ralcheva et al. (2002 a, b)* by affirming that agonistic behaviour and plasma corticosterone could be used as consistent indices of distress in poultry. That is why the birds reared indoor have received a lower score (1).

The increased aggression in the three breeds correlated to considerably higher plasma corticosterone concentrations ($P<0.05$, $P<0.01$), compared to birds reared under the organic production system. Elevated hormonal levels in response to microclimatic stressors in various bird species were reported by *Sahin et al. (2002), Sahin & Kucuk (2003), Sahin et al. (2004), Gonzales-Esquerra & Leeson (2006), Lin et al. (2006)*. According to *Popova-Ralcheva et al. (2002 a,b)* and *Mormède et al. (2007)*, blood corticosterone is a reliable indicator of the level of stress in poultry. This is also confirmed by the research of *Hocking et al. (2001)* and *Broom et al. (2001)*. Therefore, we have assess the freedom from fear and distress in birds reared indoor with 1.

The final total welfare scores of the three studied breeds of broiler breeders reared under different production systems was compared to the maximum possible score of 10 (100%). The welfare of *White Plymouth Rock, New Hampshire* and *Barred Plymouth Rock* hens reared indoor on litter was 60%, whereas for the organic production system – 90%.

Conclusion

Broiler breeders reared indoor on litter were exposed to air humidity and ammonia concentrations close to the upper allowed limits, which acted as stressors, stimulated the release of corticosterone and ultimately resulted in altered behaviour.

The better welfare score of *White Plymouth Rock, New Hampshire* and *Barred Plymouth Rock* hens reared organically (90%) is determined by the greater number of birds spending their time in dust bathing and feather cleaning as well as by fewer episodes of aggression and plasma corticosterone levels as compared to welfare of the chickens reared indoor on litter (60%).

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Ocena dobrobiti tri rase pilića (*Gallus gallus domesticus)* u različitim proizvodnim sistemima

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Rezime

Cilj procene dobrobiti živine u različitim sistemima gajenja ima veliki značaj. Konkretno organski sistemi proizvodnje ispituju se u vezi sa poboljšanjem dobrobiti živine i kvaliteta živinskih proizvoda širom sveta.

Cilj rada je bio procena dobrobiti roditeljskih jata – *White Plymouth Rock*, *Barred Plymouth Rock* (*Line F*) i *New Hampshire,* gajenih u dva sistema – podni i organski (objekat sa ispustom).

Ispitivani su mikroklimatski uslovi, ponašanje živine i koncentracija kortikosterona u krvnoj plazmi u oba sistema gajenja.

Rezultati procene dobrobiti *White Plymouth Rock*, *New Hampshire* i *Barred Plymouth Rock*, gajenih u podnom sistemu su bili 60%, dok su u organskom sistemu – 90%. Bolja ocena dobrobiti pilića koji su organski gajeni određena je na osnovu većeg broja pilića koji su provodili vreme u kupanju u prašini ($P<0.01$) i čišćenju perja ($P<0.05$) kao i manjim brojem epizoda agresije ($P<0.01$) i nivoom kortikosterona ($P<0.01$) u poređenju sa pilićima gajenih u podnom sistemu.

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EFFECT OF ANTINUTRITIONAL FACTORS AND EXTRUSION AT THE LEVEL OF CHOLESTEROL, TRIGLYCERIDES, TOTAL PROTEIN AND TESTOSTERONE IN SERUM OF BROILER CHICKENS

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Abstract: The aim of this study was to evaluate the effects of various antinutritional factors and extrusion at the level of cholesterol, triglycerides, total protein and serum testosterone in broiler chickens. The experiment was set up as four treatments with four replications, where the chickens fed diets with different soybean varieties and different way of processing. The groups were: SS-raw standard strain, SL-raw soybeans with low levels of Kunitz trypsin inhibitor, SG-standard extruded soybeans and extruded soybeans LG with low levels of trypsin inhibitors. Significantly higher cholesterol levels compared to the SS group (3,0125 mmol/l) was in SL (3,6125 mmol/l) and SG (3,8875 mmol/l) group, while in relation to the LG (3,4 mmol/l) group was higher but the difference was not significant ($p > 0.05$). Triglyceride level was highest in the LG group (1,6375 mmol/l), followed by SL (1,425 mmol/l) and SG (1,125 mmol/l) groups and between these three groups no statistically significant differences. The lowest level of triglycerides in the SS group and is significantly lower than in the other three groups. Total protein level was highest in the SL group and significantly higher than the SG and LG groups ($p < 0.05$) and highly significant difference compared to the SS group. Differences between groups SS, SG and SL are not statistically significant.The experiment confirmed that in addition to Kunitz trypsin inhibitors are heat labile and other factors that influence different biochemical parameters of blood.

Key words: soybean, cholesterol, triglycerides, testosterone, broilers

Introduction

Corn and soybeans are the main components in poultry nutrition. Soybean is one of the most common legumes in the world. Soybean is an excellent source of

protein for poultry. Grain legumes contain thousands of different proteins *(Pugalenthi et al., 2005)*. All legumes are a good source of lysine, but deficient in amino acids that contain sulfur. The high content of lysine in protein legumes is very important from the standpoint of a balanced diet and is probably more important than the total protein content, since it makes a significant addition of legume proteins in the diet with cereals, which are known for lysine deficiency *(Sadeghi et al., 2009)*. Methionine is the first limiting amino acid for poultry in soybean seed and must be added to diets containing legumes *(Carew and Gernat, 2006)*. Soy is also a rich source of carbohydrates, fiber, oligosaccharides, izoflavonida and minerals *(Refstie et al., 1998)*. Soya bean contains 19.5% crude fat and relatively high content of lipids and carbohydrates, as well as the high protein content in grain is a rich source of energy (19.4 MJ / kg) *(Siddhuraju et al., 2000)*. Value of legume protein is significantly reduced due to the presence of toxic proteins and other anti-nutritive factors (ANF), because they reduce the utilization of proteins from these grains, and limit the use of both soybeans and other legumes *(Arija et al., 2006)*. The most important factors are anti nutritious inihibitor protease: a protein with specificity for trypsin, known as Kunitz trypsin inhibitor *(Kunitz, 1945)*, and others capable of inhibiting trypsin and himotripsin, called Bowman-Birk trypsin inhibitor *(Bowman, 1944; Birk, 1961; Yen et al., 1977)*. Trypsin inhibitor activity has a detrimental effect on the utilization of protein from legumes, especially cysteine, and he is already in deficit in grain *(Kakade et al., 1969)*. Trypsin inhibitor also interferes with normal digestible protein in the intestinal tract through the inactivation of proteolytic enzymes secreted by the pancreas, and these enzymes also contain sulfur *(Kakade et al., 1969)*. Other factors to take into account the hemagglutinin or lectins *(Douglas et al., 1999)*, and goitrogens, antivitamins and indigestible oligosaccharides *(Parsons et al., 2000)*. Inclusion of raw grain foods not only a negative effect on growth of chickens, but also leads to an increase in the relative weight of digestive organs, particularly the pancreas *(Gertler et al., 1966; Arija et al., 2006, Brenes et al., 2008)*. Most of ANF are heat labile and destroyed by different thermal treatment processes nutrients, which include extrusion. Extrusion is defined as a process that is done under pressure, humidity and high temperatures. Effects of extrusion are quite prominent on the proteins, lipids and starch, by altering their physical, chemical and nutritional characteristics *(El-Khalik and Janssens, 2010)*. Exposure to high-nutrient components of temperature and pressure leads to a reduction in the number of microorganisms *(Durmic et al., 2000)*, while destroying ANF. This technology has many advantages, including a wide possibility of application, low cost operations, high productivity, energy efficiency and high quality of the resulting product *(Brenes et al., 2008)*. Adverse effects are loss of extruding thermo labile vitamins (vitamin A, E, thiamine), lipid oxidation *(Lin et al., 1997)*, reduced availability of amino acids, especially lysine, which is associated with Maillard reaction *(Moughan and Rutherfurd, 1996)*.

Another way to improve the nutritional value of soybeans is to develop varieties with low levels of Kunitz inhibitor and / or lectins. New soybean varieties with low levels of anti-nutritive factors have been developed in recent decades. *Hymowitz (1986)* found soybean with low levels of Kunitz trypsin inhibitors. The name of this cultivar is a Kunitz and she is now a commercial variety. Better results are expected when genetically improved varieties subjected to thermal treatment *(Palacios et al., 2004)*. Serbia has developed soybean varieties with low levels of Kunitz trypsin inhibitors under the commercial name "Soy Lana" and this variety was used in our expriment, while a varieties with a standard level of soybean KTI used in experiment was variety "Balkan". Total cholesterol and triglycerides are of particular importance for cardiovascular disease, especially coronary artery disease. It is known that soy proteins have a favorable effect on lowering cholesterol and triglycerides in the blood, but very little is known about the possible impact of trypsin inhibitors on these parameters.
The aim of our experiment was to determine the influence of extrusion and levels of Kunitz trypsin inhibitor on some blood biochemical parameters of broiler chickens.

Materials and Methods

A trial lasting 42 days was set up to investigate the effect of different levels of Kunitz trypsin inhibitor and extruded soybeans on blood parameters of broiler chickens. One-day old broiler chicks male hybrids Ross-308 were measured and arranged in 16 boxes, with each box were 60 chickens. The average initial weight of chicks was even and was 42.5 + / - 7 g. The experiment included four groups with four replicates. The holding system has been the floor type, with a straw mat, and food and water were available ad libitum, with a light regime of 24 hours. Temperature and air circulation are controlled and regulated in accordance with the technology for hybrid Ross 308. Dead chickens are removed from buildings daily and mortality was below 3%. The chickens were fed diets based on corn and soybeans. Starter mixture contained 23% CP and 12.65 MJ of metabolic energy and was used from 1st to 10th days. Starter mixture, where intake all chickens in the experiment and in it was included 51.77% corn, 24.46% full fat soybean meal, 15.34% soybean meal, 4% yeast, chalk, monocalcium phosphate, salt and premix. So this starter mixture was contained 23% crude protein and 12.60 MJ of metabolic energy. Experimental diet started from the tenth day, because strains with no or low levels of Kunitz trypsin inhibitors and lectins cant be successfully used in young chickhens without heat treatment *(Palacios et al., 2004)*. Experimental mixtures were grower and finisher, and differed in four groups according to whether it was included raw or thermally processed soy standard varieties (Balkan) or varieties with low levels of Kunitz trypsin inhibitor

(Lana). The mixtures used in the experiment were iso-energy and iso-protein. Chemical analysis of mixtures were made in Animal feed laboratory, Faculty of Agriculture in Novi Sad. Grower diets containing 22% crude protein was used from 11th to 24th days, and finisher with 21% crude protein from 25th to 42th days. Mixtures consisting of maize, soybean meal, dried yeast, chalk, monocalcium phosphate, salt and premix. The experimental mixture included 30% soybean and to the treatments: 1) raw soybeans with low levels of Kunitz trypsin inhibitor (SL), 2) raw standard strain (SS), 3) extruded soybeans with low levels of Kunitz trypsin inhibitor (LG) and 4) standard extruded soybeans (SG). The levels of trypsin inhibitors in soybean are presented in Table 1.

Table 1. Level tripsin inhibitor in soybean

Group Tretman	SL RKFSB	SS RCSB	LG EKFSB	SG ESBM
TI (mg/g/min)	15.07	30.21^{A}	12.43	12.30

A-values with superscript in same column are values with hi significant diference ($p<0.001$)

With 28th days of age by two chickens from box (8 per treatment) were selected and placed in a metabolic cage. Their blood was taken 32nd days from the wing vein, and before that were starved 12 hours. Blood seamples were left to stand for 2 hours at room temperature, then centrifuged at 3000 rpm at 4 ° C for 15 minutes and then left in a freezer at -18 º C until analysis. Blood serum was analyzed on the automated analyzer COBAS MIRA Plus, Rosh manufacturers and used as commercial kits manufacturers Bio-Systems (Spain) for the determination of cholesterol (colesterol oxidase / peroxidase) and triglycerides (glycerol phosphate oxidase / peroxidase), Pointe SCENTIFIC inc. (USA) for determination of total protein (Biuret color reaction). Testosterone level was determined by immunofluorescence assay (Microplate immunoassay Chemilluminescence) per manufacturer's instructions (Monobind Inc., USA). The results were tested in STATISTICA 9 (Stat Soft), we used one way ANOVA test, and for the determination of significance was used LSD Fisher test. Differences were considered significant when $p <0.05$, and together has significantly higher when $p <0.001$.

Results

Table 1 shows the levels of trypsin inhibitor content in soy and expect the highest level in the SS group and differed significantly ($p <0.001$) than the other three groups. Slightly higher levels of trypsin inhibitors in the SL grain compared with the SG and LG, but the differences were not significant. Trypsin inhibitor values being higher in LG than in SG grain, which was not significant, but are

expected to be reversed. Table 2 shows values obtained for cholesterol, triglycerides, total protein and serum testosterone in chickens. Cholesterol level was the highest in the SG group, and lowest in the group which was fed raw soybean and differences between these two treatments was statistically significant ($p < 0.05$). Significantly higher cholesterol levels compared to the SS group and in the SL group, and higher in the LG group compared to the SS group, but the difference was not significant ($p > 0.05$). Between SL, SG and LG groups no statistically significant differences in the level of cholesterol in serum. Triglyceride level was highest in the LG group, followed by SL and SG groups and between these three groups no statistically significant differences. The lowest level of triglycerides in the SS group and is significantly lower than in the other three groups. Total protein level was highest in the SL group and significantly higher than the SG and LG groups ($p < 0.05$) and highly significant difference compared to the SS group.

Table 2. Biochemical blood parameters of broiler chickens

Tretman	SS	SL	SG	LG
Cholesterol (mmol/l)	3.0125^{b}	3.6125^{a}	3.8875^{a}	3.4^{ab}
Triglyceride (mmol/l)	0.7125^{b}	1.425^{a}	1.125^{a}	1.6375^{a}
Total Protein (g/l)	33.625^{Bc}	43.3^{Aa}	35.4375^{c}	36.75^{c}
Testosterone (ng/ml)	0.270875^{b}	0.3155^{ab}	0.4935^{a}	0.3805^{ab}

A, B-values with different values in superscript in the same row are statistically highly significantly different ($p < 0.001$)

a, b, c-values with different values in superscript in the same row are significantly different ($p < 0.05$)

Differences between groups SS, SG and SL are not statistically significant, with the lowest total protein concentration was measured in the SS group. Concentrations of testosterone is highest in the SG group, followed by LG and SL groups, between which there is no significant difference, and the lowest value was measured in the SS group, where the difference was statistically significant only in relation to the SG group ($p < 0.05$).

Discussion

Extrusion significantly improves the performance, size of digestive organs and especially positive effect on the relative weight of the pancreas and digestion of food *(Arija et al., 2006.; Brenes et al., 2008)*. These beneficial effects may be associated with reduction or inactivation of lectins, trypsin, and α himotripsin amylase inhibitor *(Arija et al., 2006; Brenes et al., 2008)*. Extrusion also leads to starch gelatinisation and reduces the activity of trypsin inhibitors, which improves

the conditions for efficient digestion of proteins in the small intestine of pigs *(O'Doherty and Keady, 2001)* and chickens *(Arija et al., 2006)*. Crude genetically enhanced soybeans (Soja Lana) was as expected had a much lower level of trypsin inhibitors in comparison with the standard raw soybeans, as Kunitz trypsin inhibitor antitrypsin, main component of the seed *(Friedman et al., 1991)*. Soybean varieties with low levels of Kunitz inhibitors may have different levels of trypsin inhibitors, depending on the spot they are grown *(Kumar et al., 2003)*, the presence of other types of trypsin inhibitors *(Tan-Wilson et al., 1987)*, variations in the level lipoksigegenase *(Machado et al., 2008)* and anti-nutritive factors such as phytates and tannins *(Liener, 1994)*. *Palacios et al. (2004)* have obtained results by genetically improved strains that had better quality than standard soy protein, but *Machado et al. (2008),* in its biological assay did not perceive significant differences when the two varieties of soybean were thermally treated in the same way. This can be explained by different levels of lectins in different cultivars of soybean *(Friedman et al., 1991)*. Differences in the quality of protein are expected due to variations in the quantity of different types of trypsin inhibitors and other anti-nutritive components in different varieties of soybean *(Liener, 1994)*.

Cholesterol is the precursor of all steroid hormones and bile salts. Cholesterol levels may vary depending on the climatic conditions of the environment, nutrition, fitness broilers and sexual activity *(Itoh et al., 1998)*. Cholesterol in plasma was reduced in the experiment *Carew et al. (1998b)* in chickens that are eaten raw or heat-treated grain legumes Mucuna prurens. *Iauk et al. (1989)* showed that feeding rats with legumes leading to lower cholesterol levels. Lowered plasma cholesterol in chickens that eat raw grain legumes indicates disruption of endogenous cholesterol metabolism, which is caused by ingestion of food *(Arya, 2006)*. Similar results were obtained in our experiment when it comes to the SS group, but the unexpected result of the higher level of cholesterol in the SL group than in LG group, although the difference was not statistically significant. Protein, amino acid composition, lipid fractions, fiber and phytosterols legumes could affect the metabolic mechanisms that affect the intestinal microflora, and cholesterol synthesis and excretion of bile acids *(Martins et al., 2004)*. The results of *Martins et al. (2004)* suggest the reduction of total cholesterol in plasma of piglets that were fed raw peas. *Carew et al. (1998)* argues that the effect of legumes on cholesterol in plasma is not associated with thermo sensitive factors in raw beans.

Triglyceride levels increased with age of female Japanese quail in the paper of *Hassan (1998)*. *Arija et al. (2006)* have noted increased levels of triglycerides in groups that were fed extruded diets with a grain of beans. According to our results, triglyceride level was significantly higher in SG and LG groups fed extruded food in relation to the group which was fed raw soybeans, but unexpectedly high concentrations, even somewhat higher than in the SG group was

observed in the group fed raw soybeans with reduced levels of trypsin from soybean inhibitor.

Oligosaccharides may have significant impact on reducing levels of cholesterol and triglycerides in rats fed with high fat content *(Chen et al., 2010)*. *Huang et al. (2006)* showed that the activity of antioxidant enzymes in the liver, such as catalase, SOD and GPx were significantly increased when the soy oligosaccharides administered orally once a day to rats for 6 weeks. *Chen et al. (2010)* in *in vivo* studies have shown that soy oligosaccharides can be reduced, depending on the dose, lower oxidative stress and abnormal lipid levels in rats.

In the paper by *Arija et al. (2006)* increased concentration of grains in the mixture significantly decreases testosterone levels in broilers and this effect is attributed to the concentration of ANF in the raw grain. Besides this fact, no more information about the effects of ANF on steroid hormones in poultry. According to our results, the concentration is higher in the SS group, in which the concentration of trypsin inhibitors is higher than in the SL group, which is the level of trypsin inhibitors also decreased, while the extruded groups a higher level in the group who had higher levels of trypsin inhibitor prior to extrusion, and we can assume that if there is some anti nutritious factor that affects the testosterone, it is thermo sensitive and that was destroyed during the extrusion.

Soya bean contains isoflavonids (genistein and daidzein) that have estrogenic effects *(Messina, 1999)*. *Yousef et al. (2003)* showed a positive effect of isoflavonids on biochemical parameters of blood in male rabbits. Isoflavonids led to the lowering of cholesterol and triglycerides. In our study, the concentration of testosterone was higher in groups fed extruded diets (SG and LG), The concentration is somewhat lower in the SL group, while the lowest in the SS group, with a statistically significant difference was observed only between the SG and SS groups. The results of *Arija et al. (2006)* point to a higher level of testosterone in groups fed legumes extruded grain. Also in the the same paper extrusion of grains has led to a significant increase of the concentration of cholesterol, triglycerides, and testosterone, while the total protein had no effect. According to our results, the highest level of total protein was in the SL group and was significantly higher than the SG and LG groups ($p < 0.05$), and highly significant in relation to the SS group ($p < 0.001$). The lowest concentration was measured in the SS group. *Feng et al. (2007)* used a strain with a standard level of trypsin inhibitor and soybean, in which the level of trypsin inhibitors reduced fermentation from 2.6 to 0.0 mg / g. Measured the total protein in serum of chicks aged 21 days and 42 days old chicks. Process of fermentation and lower levels of trypsin inhibitor had no significant effect on total protein, although slightly higher concentrations observed in the group fed fermented soy. Inactivation free trypsin in the intestine stimulates the release of cholecystokinin from neuroendocrine cells in the intestine, thus leading to hypersecretion of pancreatic digestive enzymes and the consequent increase in the pancreas *(Lacourse et al., 1999)*. Cholecystokinin regulates growth of pancreatic enzyme secretion and its contraction of the gallbladder *(Rehfeld, 1998)*,

and thus the secretion of cholesterol in bile. Changes in gastrointestinal secretion affects the digestive structure and function, including a disturbance in the digestion and absorption, changes in the passage content, increased microbial activity in the small intestine and the entire increase in the digestive tract and associated organs (*Hoerr, 1998*). Size of the liver may be increased in response to several factors, especially in case of deficiency of protein and amino acids, which are associated with the presence of antinutritional factors in raw grains and legumes usually leads to accumulation of fat *(Carew and Charles, 2003)*.

Conclusion

It is possible that the availability of protein and amino acids differ among the many varieties of soybean and other legumes, which can explain the different results obtained for the impact of legumes in the diet on biochemical parameters in serum.

Our experiment confirmed that in addition to Kunitz trypsin inhibitors are heat labile and other factors that influence different biochemical parameters of blood. Extrusion process destroys antinutritive factors and improves the nutritive value of soybeans and thus also act on the aforementioned parameters. Data on the effect of content Kunitz trypsin inhibitor in soya beans on serum biochemical parameters of broiler chickens is scarce. Limited observations require more research in this area. It also lacks information about the content of other antinutritional factors in soybean "Lana" and require further investigation of the varieties and possibilities of its application in animal nutrition.

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Efekat antinutritivnih faktora i ekstrudiranja na nivo holesterola, triglicerida, ukupnih proteina i testosterona u serumu brojlerskih pilića

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Rezime

Cilj rada je bio da se ispita efekat različitih antinutritivnih faktora i ekstrudiranja na nivo holesterola, triglicerida, ukupnih proteina i testosterona u serumu brojlerskih pilića. Ogled je postavljen kao četiri tretmana sa po četiri

ponavljanja, pri čemu su pilići hranjeni smešama sa različitim sortama soje i različitim načinom obrade iste. Grupe su bile: SS-sirova standardna soja, SL-sirova soja sa sniženim nivoom Kunitz tripsin inhibitora, SG-standardna ekstrudirana soja i LG-ekstrudirana soja sa sniženim nivoom tripsin inhibitora.

Značajno viši nivo holesterola u odnosu na SS grupu je i u SL grupi, a viši je i u LG grupi u odnosu na SS grupu, ali razlika nije značajna ($p>0,05$). Između SL, SG i LG grupe nema statistički značajne razlike u nivou holesterola u serumu. Koncentracija triglicerida je najviša u LG grupi, zatim slede SL, pa SG grupa i između ove tri grupe nema statistički značajne razlike. Najniži nivo triglicerida je u SS grupi i on je statistički značajno niži u odnosu na ostale tri grupe. Nivo ukupnih proteina najviši je u SL grupi i značajno je viši u odnosu na SG i LG grupu ($p<0,05$) i visoko značajna razlika je u odnosu na SS grupu. Razlike između grupa SS, SG i SL nisu statistički značajne, pri čemu je najniža koncentracija ukupnih proteina izmerena u SS grupi.

Oled je potvrdio da pored Kunitz tripsin inhibitora postoje i drugi termolabilni faktori koji različito utiču na biohemijske parametre krvi.

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THE EFFECT OF VARIOUS LEVEL OF SKIPJACK TUNA BONE MEAL (*Katsuwonus pelamis* L) IN RATION ON BROILER CARCASS TENDERNESS AND ABDOMINAL FAT

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Abstract: Hundred day old chicks Arbor Acres CP-707 were used randomizedly in this experiment, to study the effects of various levels skipjack tuna bone meal in ration on broiler carcass tenderness and abdominal fat, and were studied for six weeks. Research using Completely Randomized Design (CRD). The dietary treatments are: R_0 basal diet as control, R_1 basal diet + 2% tuna bone meal, R_2 basal diet + 4% tuna bone meal and R_3 basal diet + 6% tuna bone meal, and each treatments were repeated five times. Results indicated that the highest carcass tenderness was get from the broiler that fed basal diet with 6% tuna bone meal (125,4 mm/g/10sec) and the lowest was get from the broiler that fed basal diet (107 mm/g/10sec). For the abdominal fat, the results is broiler that adding skipjack tuna bone meal in the ration will give more abdominal fat to the broiler (1.89% - 1.92%) versus 1.85% for basal diet.

Key words: skipjack tuna bone meal, broiler carcass tenderness and abdominal fat

Introduction

In formulating diets, it is essential to know the birds nutrient needs, and consequently the concentration of these nutrients in the various ingredients. Diets are composed of complex organic and inorganic molecules that must be reduced in size to enable absorption *(Leeson and Summers, 2001)*. According to *Arbo Acres (2009)* poor physical feed quality will have a negative impact on broiler performance. The problems with feeding broilers today is not the knowledge of optimum nutrient to use for maximum gains and feed efficiency but how to align the growth of broilers to minimize mortality and skeletal disorders to produce more saleable meat after processing. According to *Lengkey et al. (2011)*, even there are

indicated that skipjack tuna gill meal in ration has no significantly effect on broiler carcass, but it can replace the function of fish meal in the ration. Tenderness is the process of partial relaxation of the fibres. Resolution of rigor is due to enzymatis activity and physical stretching of the muscles fibres attached to bones. Tenderness is measured by use of specialized laboratory equipment or by a taste-panel *(Bell and Weaver, 2002)*. Contrary to popular belief, what the animal is fed does not directly influence tenderness. Many factor influence meat tenderness. The most important factors are genetics, age of the animal, location of the cut on the carcass, processing, method of cooking and degree of doneness *(Epley, 2011)*. According to *Widjastuti et al. (2011)*, until 6% tuna fish silage in the diet, have no significant effects, but the 4% tuna fish silage, has the best results on final body weight, carcass persentage and meat protein conversion on broiler. But, the cholesterol contents of the carcass and liver were significantly lower in Lactobacillus cultures fed broilers, but not the muscle. Supplementation of Lactobacillus culture in the broiler diets, significantly lower in fat contents of the liver, muscle and carcass *(Kalavathy et al., 2006)*. Abdominal fat of commercial broilers age of seven weeks, according to *Richardson and Mead (2006)*, in some strain are between 2.75% to 3.15% and for male (2.67%) and female (3.27%). Most poultry rations incorporate some fish meal at levels of about 2-5% of the ration; and according to Lengkey, et al (2011), for mash ration supplemented with skipjack tuna gill meal (1.89%) and crumble ration supplemented with skipjack tuna gill meal (2.08%).

Materials and Methods

One hundred day old chicks Arbor Acres CP-707 were assigned randomly and studied for six weeks. Research using Completely Randomized Design (CRD). The dietary treatments are: R_0 basal diet as control, R_1 basal diet + 2% tuna bone meal, R_2 basal diet + 4% tuna bone meal and R_3 basal diet + 6% tuna bone meal, and each treatments were repeated five times. The broiler carcass tenderness was established by meat tenderness instruments.

Results and Discussions

The effect of Skipjack tuna bone meal on broiler carcass tenderness. In Table 1, there are the results from this research of skipjack tuna bone meal in ration, to the broiler carcass tenderness. The highest carcass tenderness was get from the broiler that fed basal diet with 6% tuna bone meal (125,4 mm/g/10sec) and the lowest was get from the broiler that fed basal diet (107 mm/g/10sec).

Table 1. The effect of skipjack tuna bone meal in ration on broiler carcass tenderness (mm/g/10 sec)

Replication	R-0	R-1	R-2	R-3
I	105	114	126	137
II	107	119	106	115
III	106	118	105	121
IV	112	107	134	131
V	105	115	134	123
Average	107^{b}	$114,6^{ab}$	121^{ab}	$125,4^{a}$

Notes :R_0 basal diet as control,
R_1 basal diet + 2% tuna bone meal,
R_2 basal diet + 4% tuna bone meal and
R_3 basal diet + 6% tuna bone meal

From Table 1, adding skipjack tuna bone meal has effect to the broiler carcass tenderness. And the tenderness will rise when the skipjack tuna bone meal level percentage more higher. In R_1 (basal diet + 2% skipjack tuna bone meal), the tenderness are 114,6 mm/g/10sec, will rise when the level of skipjack bone meal are 4% (R_2 = 121 mm/g/10sec); and in R_3 (basal diet + 6% skipjack tuna bone meal) the tenderness is 125,4 mm/g/10sec; compared to the basal diet (107 mm/g/10sec).

The effect of Skipjack tuna bone meal on broiler fat abdominal. In Table 2, there are the results of the effect of skipjack tuna bone meal in ration, on broiler abdominal fat.

Table 2. The effect of skipjack tuna bone meal in ration on broiler abdominal fat (%)

Replication	R-0	R-1	R-2	R-3
I	1.80	1.87	1.89	1.94
II	1.83	1.89	1.90	1.92
III	1.85	1.87	1.87	1.95
IV	1.86	1.88	1.92	1.89
V	1.91	1.88	1.92	1.90
Average	1.85^{c}	1.89^{b}	1.90^{ab}	1.92^{a}

Notes :R_0 basal diet as control,
R_1 basal diet + 2% tuna bone meal,
R_2 basal diet + 4% tuna bone meal and
R_3 basal diet + 6% tuna bone meal

From Table 2, the average of abdominal fat are between 1.85% to 1.92%. The highest abdominal fat is from R_3 (1.92) that using 6% skipjack tuna bone meal and the lowest is from R_0 (1.85) the basal diet without skipjack tuna bone meal. It means that adding skipjack tuna bone meal in the ration will give more abdominal fat to the broiler. But this results are under the results of *Lengkey et al. (2011)* supplemented with skipjack tuna gill meal for crumble ration (2.08%) and *Richardson and Mead (2006)*, between 2.75 and 3.15%. It means that adding

skipjack tuna bone meal, is better when used in the ration, because the abdominal fat is lower than using other supplement in the ration.

Conclusion

Results indicated that the highest carcass tenderness was get from the broiler that fed basal diet with 6% tuna bone meal (125,4 mm/g/10 sec) and the lowest was get from the broiler that fed basal diet (107 mm/g/10sec). For the abdominal fat, the result is broiler that adding skipjack tuna bone meal in the ration will give more abdominal fat to the broiler (1.89 - 1.92%) versus 1.85% for basal diet.

Uticaj različitih nivoa brašna od prugaste tune (*Katsuwonus pelamis* L.) u obroku na mekoću trupa brojlera i abdominalnu mast

H. A. W. Lengkey, B. Bagau, L. Adriani, M. Ludong

Rezime

Pilići Arbor Acres CP-707 starosti sto dana su korišćeni u ovom ogledu, kako bi se ispitali uticaji različitih nivoa brašna od prugaste tune u obroku na mekoću trupa brojlera i abdominalnu mast, u istraživanju koje je trajalo 6 nedelja. Istraživanje je izvedeno korišćenjem - Completely Randomized Design (CRD). Hranidbeni tretmani su bili sledeći: R_0 bazalni obrok kao kontrola, R_1 bazalni obrok + 2% koštanog brašna od tune, R_2 bazalni obrok + 4% koštanog brašna od tune i R_3 bazalni obrok + 6% koštanog brašna od tune, i svaki tretman je ponovljen pet puta. Rezultati ukazuju da je najveća mekoća trupova dobijena kod brojlera koji su hranjeni bazalnim obrokom sa 6% koštanog brašna od tune (125,4 mm/g/10sec) a najniža kod brojlera hranjenih bazalnim obrokom (107 mm/g/10sec). Za abdominalnu mast, dobijeni rezultati pokazuju da dodavanje koštanog brašna prugaste tune u obrok će rezultirati u povećanju abdominalne masti kod brojlera (1,89% - 1,92%) prema 1,85% kod bazalnog obroka.

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THE NUTRITIVE VALUE OF EXTRUDED CORN IN NUTRITION OF BROILER CHICKENS

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Abstract: In poultry farming is increasing effort to increasing profitability and decreasing of feed cost. Behind the components that are part of the mixture, the most of cost makes processing of nutrients. Extrusion is process which is increasingly used in order to enhance the quality of different feed for poultry. The aim of this research was comparative testing of nutritional value of extruded and nonextruded corn in nutrition of broiler chicken. The experiment was conducted under experimental growing conditions, in duration of 42 days. The following parameters were measured: growth, feed intake and vitality of broilers. The experiment was divided into two groups of chickens hybrid Ross-308, which were grown separately by sex with 8 replication. The first group was fed standard pelleted mixtures for growing chickens, in which the entire amount of corn in the mixture was extruded, and the second, control group was fed the same food in which corn was not extruded. In the second week of rearing the body weight was 437 g in both groups. In 4th week of age the average body weight was 1439g in group I, and 1419 in group II. For the first group conversion was 1,69 and for the second it was 1,71. The final body weight amounted 2595g for the first group and 2609 for the control group. In the first group conversion was 1,91, mortality 4,39% and the production index had a value of 309, and in the second 1,92; 4,56% and 306. In this study excellent production effects were achieved, so it was not expected that extrusion could achieve better production effects. These results showed that extrusion process does not always give the expected effects.

Key words: extrusion, corn, nutrition value, broiler chickens

Introduction

Food is the most significant cost in raising chickens. Definitely, feed ingredients make up the largest portion of the cost of food. However, the cost of processing food are an important part of the cost of feeding broilers and processing, ina addition to food ingredients, provides the best opportunity to influence the performance of broilers *(Milošević et al., 2006; Wu and Ravindran, 2004; Gracia*

et al., 2009; Beuković et al. 2010a). Almost every food processing has a positive or negative effect on animal performance and can certainly affect the profitability of production *(Buchanan et al., 2010)*. Improving the nutrional value of maize is important, since corn is the dominant energy source in poultry nutrition *(Milošević et al., 2006)*. Its high starch content, high content of oil and a bit of cellulose, makes the corn highly energetic feed which organic matter have digestibility of 90%. The amount of individual components varies depending on the type of hybrids and growing condition *(Jeličić et al., 2009)*. In recent years the extrusion increasingly used in food for animals, because this technology has numerous advantages, including the possibility of wide application, high productivity, energy efficiency and high quality of the resulting product *(Brenes et al., 2008)*. Extrusion of corn may increase digestability of starch, since starch is becoming more accessible to digestive enzymes, resulting in greater mass of chicks *(El-Khalek and Jamssens, 2010)*. Extrusion leads to the formation of complex fats and carbohydrates and enhances stability; prevents oxidation process over inactivate lipolytic enzumes *(Strugar et al., 2006)*. Besides the positive effects of extrusion of maize, in previous research researcher were also found some negative consequences. In the work of *Moritz et al. (2005),* process of extrusion of maize reduced the nutritive value of maize in the diet of broilers aged from 0 to 3 week. *Amornthewaphat et al. (2005)* have observed that extruded crushed corn has a negative effect on performance of broilers. The aim of this study was comparative testing of nutrional value of extruded and nonextruded corn in the diet of broiler chickens.

Materials and Methods

Day-old chicks hybrid Ross-308 were separated by sex with method based on the length of cover and primary wing feathers. The experiment was set up as two treatments with eight replications. The first group was fed standard diets for fattening of broiler chickens (starter, grower and finisher), in which the entire amount of corn in the mixture was extruded. The second group (control group) was fed the same mixture in which corn was not extruded. All mixtures were pelleted. Composition of the mixture and its chemical composition are shown in Tables 1 and 2. Food and water were provided *ad libitum* during the fattening. Control measurement of chicken and food was carried out every week of experiment and a measurement of all broilers and feed was done at 2^{nd},4^{th} and at the 6^{th} week of age. Health status was monitored daily, and mortality and dead birds were regulary put away from the object. All obtained results were processed using standard statistical-variation methods (descriptive statistics, variance analysis, t-test) in the program STATISTIKA 10 *(StatSoft, 2010)*.

Table 1. Ingredients of mixture

Ingredients	Starter (0-10 day)	Grower (11-28 day)	Finisher (29-42 day)
Corn	47,36	51,93	58,14
Wheat meal	9,00	6,00	5,90
MethioninDL 99%	0,10	0,00	0,00
Soybean meal 44%	22,48	16,12	10,77
Soybean croup	16,75	22,35	21,72
Lysine L (78%)	0,19	0,00	0,00
Monocalcium phosphate	1,15	1,01	0,92
Threonine L (98%)	0,09	0,00	0,00
Salt (NaCl), iodized	0,33	0,33	0,33
Limestone	1,56	1,26	1,22
Premix+enzyme supp.	1,00	1,00	1,00

Table 2. Chemical composition of mixture

Chemical composition	Starter (0-10 day)	Grower (11-28 day)	Finisher (29-42 day)
Dry matter %	89,54	89,52	89,50
Crude protein %	22,00	21,03	19,00
Crude fat %	5,44	6,43	6,50
Crude ashes %	6,25	5,62	5,20
Crude cellulose %	4,36	4,08	3,82
ME (MEn) MJ/kg	12,65	13,20	13,40
Lysine %	1,43	1,24	1,10
Methionine %	0,72	0,61	0,59
Methionine + Cystine %	1,07	0,95	0,90
Threonine %	0,94	0,84	0,75
Tryptophan %	0,30	0,28	0,24
Ca %	1,05	0,90	0,85
P (total) %	0,81	0,76	0,76
P (useable) %	0,50	0,45	0,42
Na %	0,16	0,24	0,16
Cl %	0,27	0,24	0,25
C18:2 (linoleic) %	2,78	3,28	3,30
Xantophyll mg/kg	8,01	8,78	9,83

Results and Discussion

The average body weight of broiler chickens at 2^{nd}, 4^{th} and 6^{th} week of age are shown in Table 3.

Table 3. Average body weight, g

Age, weeks	Group	
	Extruded	Control
2	437	437
4	1439	1419
6	2595	2609

From the results, we can see that the extrusion of corn had no effect on body weight of chickens. The slightly higher weight of chickens was observed in 4th week old, but the difference was not statistically significant. At the end of fattening weight of broilers was higher in the control group, but the difference was not statistically significant. In both groups achieved excellent production results when it comes to body weight. Health and vitality of broilers were also at a high level in both groups. The results of feed conversion at the 4th and 6th week of age, mortality and value of European Production Efficiency Factor (EPEF) are shown in Table 4. Slightly higher conversation was observed in the control group and the fourth and the sixth week of experiment. Mortality, expressed as a percentage was slightly higher in the control group, while the value of EPEF was higher in the group that was fed extruded corn. The observed differences were not statistically significant.

Table 4. Feed conversion at 4 and 6 week, mortality and European Production Efficiency Factor

Feed conversion and EPEF	Group	
	Extruded	Control
Feed conversion at 4 week	1,69	1,71
Feed conversion at 6 week	1,91	1,92
Mortality, %	4,39	4,56
European Production Efficiency Factor (EPEF)	309	306

The results indicate that the extrusion of corn had no effect on the performance of broilers. Previous research on the effects of extrusion on the performance of poultry have been inconsistent results *(Plavnik and Sklad, 1995; Amornthewaphat et al., 2005; Beuković et al., 2010b)*. *Moritz et al. (2005)* were obtained that extrusion process of corn has led to an increase in body weight of broiler chickens, aged 0 to 3 weeks. In the work of *Amornthewaphat et al. (2005)*, extruded crushed corn has a negative effect on performance of broilers. Their

results indicated that this addition to weight gain and feed conversion in poultry can be improved if the extruded corn would be pelleted. According to *Milošević et al. (2007)* extrusion of corn meal has significantly affected the improvement of production performance of broiler chickens. During the extrusion process occur nutritional changes which may be significant for the quality of extruded feed. *Moritz et al. (2005)* attributed the different effects of extrusion to different conditions during extrusion process, variations in the different effects of extrusion to different conditions during extrusion process, variations in the extrusion process that can lead to the improvement of the nutritive value of feeds, but also to its reduction. Undesirable effects of extrusion cooking involve losses of heat-labile vitamins (A, E, thiamine), oxidation of lipids *(Lin et al., 1997)*, destruction and reduction in the availability of amino acids, in particular, of lysine which is related to the Maillard reaction *(Moughan and Rutherfurd, 1996)*. As a result of the Maillard reaction, which occurs during the extrusion process, lysine is damaged and protein digestability decreases *(Hurrel and Carpenter., 1981)*. Starch is a dominant nutrient in maize. Starch plays a key role during extrusion, and undergoes several significant structural changes, which include starch gelatinisation, melting and fragmentation. Starch in grains is also an important binding agent in steam-pelleted and extruded feeds *(Jovanović et al., 2009)*. Extrusion process can reduce the availability of non starch ingredients in corn. Moisture content of diets may also influence feed intake *(Moritz et al., 2001, 2002)*. However, moisture percentages among groups were relatively similar. One explanation for the variation in results is that these differences are due to different types of extruders (wet or dry, with one or two extractors) and different condition during the extrusion process, such as temperature, humidity, pressure, etc. *(Hongtrakul et al., 1998)*.

Conclusion

As a whole, based on the examinations and the results obtained, it can be concluded that this type of research is justified because they show that the extrusion process does not always have positive effects. Considering that each processing increases the cost of food, it is not always justifiably subjected to thermal treatment of food. This does not mean that the extrusion process should be abandoned as a useless process, but should seek the causes of bad effects. In the research we have achieved excellent production effects in both groups, and there was no expectation that the extrusion process will lead to major positive effects.

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Nutritivna vrednost ekstrudiranog kukuruza u ishrani brojlerskih pilića

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Rezime

U živinarskoj proizvodnji se sve veća pažnja posvećuje rentabilnosti proizvodnje i smanjivanju troškova ishrane. Posle komponenti koje su sastavni deo smeše, najveći deo troškova otpada na proces obrade hraniva. Ekstrudiranje je proces koji se sve više primenjuje u cilju da se poboljša kvalitet različitih hraniva za živinu. Cilj rada je bilo uporedno ispitivanje nutritivne vrednosti ekstrudiranog i neekstrudiranog kukuruza u ishrani brojlerskih pilića. Ogled je izveden u eksperimentalnim uslovima gajenja, u trajanju od 42 dana. Praćeni su sledeći parametri: prirast, utrošak hrane i vitalnost brojlera. U ogledu su bile dve grupe pilića hibrida Ross-308, koji su gajeni odvojeno po polu sa 8 ponavljanja. Prva grupa je hranjena sa standardnim peletiranim smešama za tov pilića, u kojima je celokupna količina kukuruzne prekrupe u smeši bila ekstrudirana, a druga, kontrolna grupa istom hranom u kojoj kukuruzna prekrupa nije bila ekstrudirana. U drugoj nedelji telesna masa je iznosila 437 g u obe ispitivane grupe. Sa 4 nedelje starosti, telesna masa je bila 1439 g u I grupi i 1419 g u II grupi. Konverzija je u četvrtoj nedelji starost bila 1,69 (I), odnosno 1,71 (II). Završna telesna masa je iznosila 2595 g u I grupi i u II 2609 g. U prvoj grupi konverzija je iznosila 1,91, mortalitet 4,39% i proizvodni indeks je imao vrednost 309, a u drugoj 1,92; 4,56% i 306. U slučaju ovog istraživanja postignuti su vrhunski proizvodni efekti, pa nije bilo ni za očekivanje da će se procesom ekstrudiranja postići bolji proizvodni efekti. Dobijeni rezultati ukazuju da proces ekstrudiranja ne daje uvek pozitivne efekte.

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CHALLENGE FOR THE REVITALIZATION OF TRADITIONAL PRODUCTION: CONTROL OF NEWCASTLE DISEASE IN BELGRADE DISTRICT

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Abstract: The global situation of Newcastle Disease (ND) is characterized by enzootic infection in many developing countries where village chickens are considered to be the main reservoir of the virus *(Awan et al., 1994)*. Newcastle disease has been eradicated in most countries, but in developing countries occasional outbreaks occur and are mandatory reported to OIE. Development of new technologies in production of eggs of special, guaranteed quality by using hens housed in free ranges is becoming very popular trend in our country *(Pavlovski et al., 2010)*. In order to further develop such practice our country must be free of ND. The latest notifaction of ND in Serbia and Belgrade, was in the period from 2006 to 2007. Newcastle disease virus (NDV) caused sporadic outbreaks among non-vaccinated domestic fowl (backyard chickens). The comparative serological data prior to the outbreak (in one district) has shown high titers in unvaccinated backyard flocks. This finding suggested that the virus was not dormant. The distribution of the high antibody titers tends to suggest close contact with infected birds and/or other means of virus transmission that contributes to ND spreading. In the Republic of Serbia preventive vaccination is mandatory for all commercially raised chickens, turkeys, game birds raised in farms and carrier pigeon participating in exhibitions. Control of effectiveness of the vaccine is regularly performed, only in commercial chicken flocks. A serological survey of domestic fowl is done periodically. After the outbreak occurred, small chicken flocks (less then 350 chickens in the household), were vaccinated with La Sota strain. Subsequently the blood samples were collected and investigated in order to estimate the level of protection and to identify vaccinated birds. Continual vaccination programs currently offer the only sustainable prospect for control of ND. The authors give information on the NDV antibody status of the poultry population in one Belgrade district estimated by recommended serological tests.

Key words: backyard chickens, Newcastle disease, outbreaks, serological control.

Introduction

Newcastle disease (ND) is a disease of major importance for poultry and other birds. It is caused by specified viruses of the avian paramyxovirus type (APMV-I) of the family *Paramyxoviridae*.The taxonomy and nomenclature of the family *Paramyxoviridae* was modified and it is now divided into two subfamilies. The subfamily *Paramyxovirinae* has three *genera:Respirovirus, Morbillivirus and Rubulavirus* which include the Newcastle disease virus (NDV*.)* Paramyxoviruses characteristically consist of a single molecule single-stranded RNA *(Saife et al., 2003; Calnek, 1997)*. There are three categories of Newcastle disease virus (NDV) velogenic (high virulence), mesogenic (moderate virulence) and lenotogenic (low virulence) based on chicken embryo mortality at <60hr, 60-90hr and >90hr respectively, after allantoic inoculation *(Hanson and Brandly,1955)*.

The disease is characterised by respiratory and/or nervous signs, partial or complete cessation of egg production or misshapen eggs, greenish watery diarrhoea and oedema of the tissues around the eyes and the neck. The infection is spread via direct contact with secretions, especially faeces, from infected birds or indirect contact through contaminated feed, water, equipment, vehicles, humans, fomites etc.

The use of prophylactic and emergency vaccination is permitted in Serbia as well in EU. Newcastle disease is a compulsory notifiable disease in Serbia listed also by the World Organisation for Animal Health *(OIE, 2004)* and due to its potential for very serious and rapid spread, irrespective of national borders with serious socio-economic consequences requirements for international trade of live animals and animal products are laid down in the Terrestrial code.

The global situation of ND is characterized by enzootic infection in many developing countries where village chickens are considered to be the main reservoir of the virus *(Awan et al., 1994).* Newcastle disease has been eradicated in most countries, but in developing countries occasional outbreaks occur and are mandatory reported to OIE. In order to develop and revitalize traditional free rearing of poultry, in poultry industry it is very important to monitor NDV presence applying regular serology testing of all poultry flocks and other birds that are in contact to humans and chickens. The particular problem in Serbia is backyard chicken flocks since these birds are not always protected against Newcastle disease and may present virus reservoir for long.

In this paper serology monitoring before and after an outbreak of ND in Belgrade district in the year 2006-2007 is described. Belgrade is the capital of the Republic of Serbia. In Belgrade districts there are 12.806 backyard chicken flocks recorded in Central data base of Veterinary Directorate. At that time regular

vaccination against ND was not done on all of those holdings and subsequently some birds were infected with the wild type virus.

Possible reasons for the virus spread in the district of Belgrade are briefly discussed.

Materials and Methods

Backyard chickens (domestic poultry) in Belgrade district. Prior to the sporadic ND outbreak in the Belgrade district, studies on 690 blood samples originating from non-vaccinated birds from fowl grown in villages for own , non-commercial consumption of meat and eggs were carried out.

After notification of few outbreaks of ND in Serbia, Veterinary Directorate enforced strict control measures on the territory of the whole country, including registration of poultry holdings (including backyard ones) in Central database and vaccination of whole poultry population .

In order to verify the quality of performance of such measures, veterinary service organized blood sampling on different locations in the district and detection of the presence of antibodies against NDV.

In the first stage of the study a total of 900 blood samples of vaccinated birds were analyzed.

Detection of NDV antibodies. To control the antibody titer, the haemagglutination inhibition test (HI-test) with La Sota antigen was used according to *(OIE Manual, 2004)*.

Results and Discussion

A few months before the ND was registered, control of serum samples originating from non-vaccinated small floks of domestic layer chickens of different ages from an area close to the zone where ND appeared was carried out. These birds presented hazard to breeding farms. Out of the 690 studied blood samples only 4.84% were tested negative for ND virus. Results are given in Table 1.

Table 1. Results of antibody testing in the Belgrade district before ND outbreak

Tested Flocks in Belgrade District	Nº of sera	Results of serology testing		Nº of Flocks
		NDV antibody	*NDV antibody*	
		+	-	
Domestic small flocks	690	620	70	29

In each studied household (29), in the Belgrade district only layer chickens were held. Mean HI titers for each household are presented in Table 2.

Table 2. Mean HI titer before ND outbreak in Belgrade and number of birds/household tested

No birds of tested	Mean HI titres (log^2)	No of birds tested	Mean HI titres (log^2)	No of birds tested	Mean HI titres (log^2)	Age of the layers (years)
64	3.4	17	2.5	36	3.7	1-2.5
29	2.8	16	3.1	13	2.6	
33	1.8	14	4.0	17	0.2	
15	2.3	21	4.9	45	5.9	
27	4.0	24	2.5	9	0.3	
24	1.4	14	2.0	7	3.1	
19	4.3	13	2.7	29	5.5	
13	0.2	10	2.8	38	2.8	
30	3.1	25	3.0	50	3.5	
21	5.8	17	4.1			

According to the Project funded by the Ministry of Agriculture and Forestry and Water Management; Veterinary Directorate of the Republic of Serbia ND vaccination of small domestic flocks was introduced in 36 households in Belgrade. The sera were collected from vaccinated birds 2-3 weeks post vaccination and the obtained results are shown in Table 3.

Table 3. Number of samples, number of flocks and HI test results after vaccination

Nº of sera	Serology results		Nº of households
	NDV antibody	NDV antibody	
	+	Mean HI titers Log_2	
900	900	4.9-5.1	36

The number of samples per holding was collected according to the number of birds, thereafter the sample size was between 23 and 27. The mean HI antibody titers were in the interval from 4.9 to 5.1.

In our study, we have found NDV antibodies within the unvaccinated backyard chicken flocks in Belgrade districts. This means that, small poultry flocks were in the contact with the wild type ND virus and subsequently the outbreak occurred. In Serbia vaccination of fowl, farm pheasants and pigeons with lentogenic vaccine strains is compulsory.

Until 2007 serology control of backyard chicken flocks was done occasionally in spite of the strict legal provisions , while in large industrial poultry farms NDV immunity is investigated, by regular serological testing.

Newcastle disease outbreak in backyard poulty flocks in the district of Belgrade was reported to OIE *(WAHID, ND Serbia follow up report, 2006)*.

Subsequently, the Ministry of Agriculture, Forestry and Water Management introduced in Serbia improved control measures in poultry production also in village chicken flocks. Despite the difficult socio-economic situation in the country, the State Budget has financed vaccinations in backyard flocks with La Sota vaccine on two occasions during 2007.

Many efforts have been made in Serbia to establish NDV immune status and to collect epizootiological data including the number of households keeping animals, number of flocks, flock size and other, in order to design an efficient monitoring system. Since there were no reports of NDV in the country after year 2007 we concluded that improvement in raising backyard chickens is evident.

There are many ways of NDV transmission over aerosol of infected birds or if the people and equipment become vehicle for the virus spread. It is our opinion that the virus spreading over exotic birds, raising pigeons and game birds in Belgrade is possible . In Epidemiological study of ND in backyard poultry and wild bird populations in Switzerland, *(Shelling et al., 1999)* have described the existence of NDV antibodies in water birds *Tachybaptus ruficollis* and *Phalacrocorax carbo* in 100% and 18.9% samples, respectively.

In the same study in wild birds, such as the Eagle Owl (Bubo bubo) and the Sparrow hawk (Accipiter nisus) the incidence was 100% and 58.8%, respectively. The most common wild birds discovered in the studied backyards were birds of pray (70%) and crows (15%). This means that transmission of NDV in the nature is possible and that there are no strict ecological boundaries when it comes to ND. Birds in migration know no borders and they carry on their feathers everything present in their environment, either on land or in air.

Kaleta and Baldauf (1988) reported that NDV has been isolated from up to 240 different birds species. It is believed that virtually all known 8000 birds species may become infected and shed NDV, while clinical signs in wild birds populations have been rarely reported.

Due to an increasing international movement of humans and animal commodities, including import of foreign and exotic birds and food of poultry origin in our country, quarantine for imported birds needs to be carefully implemented since trade with such birds is common in the Belgrade region as well

in other regions in the country. Parrots and other psittacine birds are especially dangerous because they can carry exotic Newcastle disease virus and show no signs of disease.

In the period 1984-1995 the analysis of genetic characteristics of ND in the region was conducted *(Wehmann et al., 2003)*. Total of 29 isolates from the territories registered to have ND in Serbia were tested. Isolates of ND viruses were named after places, but they also have indication of ditrict area where ND appeared most often. Based on diagnostic examinations and reported cases on the territory of Belgrade ND was registered *(Blažin et al.,1988)*, as well as in 2002 (notified case), both times on the territory of Kovačevac, as well as in 2006 on the territory of Koraćica-Kosmaj. Testing of several field ND isolates in Serbia based on chicken embryo mortality indicated the presence of velogenic and mesogenic type virulence *(Miljković et al., 1992)*. An experience in previous period shows that the selection of place/location for free rearing of chicken in relation to incidence of ND should not be in the region where poultry is reared intensively, Mladenovac and area in its vicinity.

In 2011 the implementation of the Rulebook on program of measures of animal health protection started, Official Journal of RS RS 24 /11, stipulating that poultry, game birds and pigeons, in all housing systems, must be vaccinated against ND using the lentogenic strain vaccine in order to achieve permanent level of immune protection. Serological controls of all categories of reared poultry are mandatory.

Conclusion

The use of prophylactic and emergency vaccination is permitted in Serbia as well in EU. Continual vaccination programs currently offer the only sustainable prospect for control of ND. It is strongly recommended to establish effective surveillance system of the poultry flocks, pigeon and game birds which should be monitored by veterinary service.

In selection of the place/location for development of traditional free poultry rearing, the areas without intensive poultry production should be selected, territories of hilly configuration and lower population of village poultry. Stated serological results obtained in the control of backyard chickens can be used as guide in indirect detection of the presence of field virus.

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Izazov za revitalizaciju tradicionalne proizvodnje: serološka kontrola Newcastle bolesti u Beogradskom okrugu

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Rezime

U mnogobrojnim zemljama u razvoju, globalna situacija Njukasl bolesti (Njb) se karakteriše enzootskom infekcijom, gde je seoska živina svakako glavni rezervoar virusa *(Awan et al., 1994)*. Ona je eradicirana u mnogim razvijenim zemljama, ali u zemljama u razvoju česte su sporadične pojave koje se obavezno prijavljuju OIE. Razvijanje novih tehnologija za proizvodnju jaja posebnog garantovanog kvaliteta sa kokicama koje su slobodne u ispustima, postaje veoma popularni trend u našoj zemlji *(Pavlovski et al., 2010)*. U cilju razvijanja takve proizvodnje naša zemlja mora da bude slobodna od ND. Poslednja prijava ND U Srbiji i Beogradu, bila je u periodu od 2006 do 2007. Virus (Njb) iznenada se sporadično pojavio kod dvorišne (domaće živine) u Srbiji, a i na jednom distriktnom području u Beogradu. Komparativni serološki podaci pre pojave bolesti (u jednoj zoni distrikta) ukazali su na postojanje visokog titra antiitela kod nevakcinisanih seoskih jata živine. Ovaj nalaz je ukazivao da virus nije bio skriven. Distribucija visokog titra antitela pokazala je da je živina bila u bliskom kontaktu sa virusom a to je i glavni mehanizam širenja virusa Njb. U proteklom periodu preventivna vakcinacija je obavezna kod svih komercijalnih jata pilića u odgoju, domaće živine, ćurića, fazana i golubova koji su u prometu. Kontrola efikasnosti sprovedene vakcinacije je obavezna jedino, kod komercijalno gajene živine. Domaća živina se ciljano ispituje samo periodično. Nakon sporadične pojave Njb, mala jata živine (do 350 pilića po domaćinstvu) bila su vakcinisana sa La Sota vakcinom, a nakon toga su uzorci krvi sakupljeni radi utvrđivanja nivoa zaštite. Kontinuirani vakcinalni program i uspostavljanje barijere imune zaštite su mere koje se primenjuju u kontroli bolesti. Autori saopštavaju vrednost titra antitela Njb kod ispitane populacije živine u distriktu Beograd, na osnovu preporučenog serološkog testa.

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THE EFFECT OF FEEDING FERMENTED KOMBUCHA TEA ON HDL, LDL AND TOTAL CHOLESTEROL LEVELS IN THE DUCK BLOODS

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Abstract: Kombucha have high content of glucoronic acid, a substance that could neutralized cholesterol deposit, changed to another compound that more soluble in water. The experiment concerns the effects of supplementary fermented kombucha tea on HDL, LDL, and total cholesterol levels in the duck bloods. It was carried out at the Faculty of Animal Husbandry, Universitas Padjadjaran. The objective of this research was to determine the effect of giving different levels of fermented kombucha tea on High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and the total cholesterol levels of duck bloods. This research used an experimental methods with completely randomized design (CRD). There were five treatments (P0 = 0%, P1 = 10%, P2 = 15%, P3 = 20%, and P4 = 25%) and four replications. Results indicated that all treatment decreased significantly LDL and total cholesterol levels, and increased HDL. The conclusion is, the addition of fermented kombucha tea up to 25% will decrease LDL and total cholesterol, and increase HDL in the duct blood.

Key words: kombucha fermented tea, HDL, LDL, total cholesterol, duck blood

Introduction

Kombucha is fermented tea that is often drunk for medicinal purposes. There are scientific studies that support the health benefits of Kombucha that show it to be antimicrobial *(Sreeramulu et al., 2000; Cetojevic-Simin et al., 2008)*, have hepatoprotective qualities (Murugesan et al,2009) and to be antioxidative *(Sai Ram et al., 2000; Dipti et al., 2003)* among other benefits. Kombucha is available commercially or can be made at home by fermenting tea using a visible, solid mass of yeast and bacteria. Kombucha contains multiple species of yeast and bacteria, as well as the organic acids, active enzymes, amino acids, and polyphenols produced by those microbes. Many health benefits have been reported by users of Kombucha

Tea, benefits are derived at due to its cleansing properties by detoxifying and aiding the liver and kidneys to flush the toxins from the body. The health benefits of this living beverage are varied. Efforts to reduce Total Cholesterol, HDL, and LDL in the duck can be done with giving Kombucha Tea Fermentation (TFK) in duck drinking water. Fermented kombucha tea can be consumed as a food supplement that offers the required compounds in stabilizing the body's metabolism. According to *Williams (2001)*, yeast ferments contained in kombucha tea is *Candida albicans*, *Sacharomyces*, and *Pichia fermentans* while the bacterium *Acetobacter xylinium*, *Gluconicum bacteria*, *Acetobacter ketogenum*. The suspension is glucoronic acid, gluconic acid, lactic acid, oxalic acid, lactic acid, butyric acid and natural antibiotics material. In addition to producing some organic acids . Kombucha also produce some vitamins such trace element that give a benefit for health body. The vitamin B groups in kombucha have a function for regenerating energy and metabolizing lipid and protein. Besides that it is also important for the nerve system. Vitamin C in kombucha have a potency as detoxifier agent, and it also able to support immune system, and increase the vitality *(Anonymous, 2006)*. Glucuronic acid also present in kombucha tea, this acid is a metabolite that is produced by a healthy liver and aids in the detoxification of the body. By drinking kombucha tea daily will help prevent our body tissues from absorbing all the toxins found in our industrial environment that can lead to illness *(Naland, 2008)*. Kombucha tea contains most polyfenol, including flavonoids. One of the flavonoids is catechin, these compounds are antioxidants with the power 100 times higher than vitamin C and 25 times than vitamin E, which is also a powerful antioxidant. Changes in LDL (Low Density Protein) into a form that LDL oxidized by free radicals can cause damage artery walls and increases atherosclerosis violence. Prevention mechanisms contained in its ability to inhibit the absorption of cholesterol and inhibits platelet clumping cells thus preventing the blockage of blood vessels. Polyfenol tea is also a powerful antioxidant that can protect LDL oxidation by free radicals.

According to *Mohan (1996)* and *Santoso (2000)* the addition of products fermented can decrease cholesterol levels through the mechanism of inhibit HMG CoA reductase enzyme activity (3-hydroxy 3-metilglutaril CoA reduckase) as a producer or through the mechanism of increased cholesterol synthesis bile acids. Increased secretion of bile acids will increase the excretion of cholesterol so that cholesterol levels in the blood decreased *(de Roos and Katan 2000)*. Reducing cholesterol levels due to inhibit mechanism of synthesis of HMG CoA played by the components contained in Kombucha tea. As it is known that HMG CoA could form mevalonat acid compound which is a precursor of cholesterol *(Martin et al., 1981)*.

The Kombucha colonies used in this investigation had a tendency to produce about 3.3% total acid, 0.7% acetic acid, 4.8% glucose, and 0.6% ethanol after a nine-day fermentation. There was no lactic acid produced by these colonies

(verified with HPLC; 9). The average pH of the fermented samples tested was 2.5. The pH of the neutralized samples was 7.0. When the fermentation was allowed to continue beyond the desired endpoint, the acidity reached levels as high as 24 g/L (2.4%) acetic acid, with 14 g/L (1.4%) ethanol.

Materials and Methods

Kombucha that have high level of glucoronic acid, would be test to decline blood cholesterol. Twenty ducks, with average body weigh 1800 gram and coefficient variable 8,59%, age 1,5 years The duck kept in the cage, as much as 5 group, and each of it contain 4 duck.
The ration consist 16% protein and metabolist energy 2900 kcal/kg
The formula rations were **:**

R0 Control diets
R1 Diets contain 10% of kombucha
R2 Diets contain 15% of kombucha
R3 Diets contain 20% of kombucha
R4 Diets contain 25% of kombucha

The dosage of kombucha (10, 15, 20, 25%) from drinking water has been standardize according to water consumption in premilary experiment. The range of temperature was between 24 until 32^{O}C.

Sample preparation: Kombucha was prepared by adding 100 g/L (10%) weight/volume sucrose and tea leaves of desired dry weight to boiling water. The fermentation time averaged twelve days at 25° C *(Lovita et al., 2011).*

Results and Discussion

Table 1. Effect on Total Cholesterol, HDL and LDL (Mg/dl) of duck blood

Variables	P0	P1	P2	P3	P4
Total Cholesterol	213,23	194,10	183,67	172,60	164,78
LDL	54,53	40,23	31,92	24,14	19,43
HDL	61,99	80,19	85,17	93,15	123,85

Effect treatment on total cholesterol levels of duck blood. Based on Table 1, Blood cholesterol in tested animal decline during treatment with consuming kombucha.. Adding 25% kombucha tea of the total drinking water consumption could be reduced highest the total cholesterol level.

Decreasing total cholesterol in all treatment, because of the catechins contained in the fermented tea Kombucha. Catechins reduce the accumulation of cholesterol in the blood and accelerates the elimination of cholesterol through the feces, as well as free radicals *(Anonymous, 2001)*. It could lower the absorption of fat and cholesterol in the intestine so that it will stimulate the secretion of bile acids were digest more fats *(Purnawan, 2010)*. Soluble fiber component also plays a role in lowering total blood cholesterol, it contained in fermented Kombucha Tea role in reducing the absorption of fat and cholesterol in the intestine, thereby reducing the cholesterol content in blood *(Kusnandar, 2004)*. In addition, soluble fiber stimulates the liver to release more bile salts into the duodenum to the liver needs cholesterol to produce more bile salts by taking cholesterol in tissues *(Astuti, 2004)*. The mechanism of decreasing total blood cholesterol is also associated with niacin, it inhibit reform and reducing fat tissue retrieval of free fatty acids by the liver so that the synthesis of cholesterol in the liver is reduced, and circulating cholesterol into the body tissues will decrease *(Naland, 2008)*. This is supported by previous research that the administration of niacin in chickens up to 4% in the ration may lower cholesterol levels in chickens due to an increase in lipolysis process so that acetyl CoA is formed from the process of beta oxidation of fatty acids declined . Glukoronat acid contained in fermentation of Kombucha tea is also a role in lowering cholesterol, that bind toxins, heavy metals and excess fat and cholesterol are easily soluble in water and excreted by the body along with urine *(Greenwalt, 1999)*. This is consistent with *Rahayu (2005)* which consume fermented Kombucha tea can reduce cholesterol level.

Effect Treatment on HDL and LDL in duck blood. HDL and LDL in cholesterol are soluble in water and could not distributed in the body. Cholesterol is distributed in blood systems by lipoprotein-protein component soluble in water which able to bring cholesterol and triglyceride in it. Cholesterol brings into the pheripherial system by cilomicron lipoprotein, VLDL and LDL. The capability of the lipoprotein to bind blood cholesterol are very low. Therefore, this kind of lipoprotein called bad cholesterol. Besides that, HDL particle have higher capability to bind cholesterol and bring it to liver then excreted to urine *(Anonymous, 2008b)*, HDL cholesterol in blood plasma of tested animal showed significantly increase during kombucha consumption. HDL cholesterol for P0, P1, P2, P3 and P4 were 61.99, 80.19, 85.17, 93.15, and 123.85 mg/dl respectively. The LDL in all treatment is decline.. LDL cholesterol for all treatment (P0, P1, P2, P3, P4) were 54.53, 40.23, 31.92, 24.14, and 19.43 respectively.

Cholesterol levels decreased because of the active substances activity that contained in kombucha tea. The addition of fermentation products could decrease the cholesterol levels of blood through the mechanism of inhibition of an enzyme activities that involved in cholesterol biosynthesis (3-hydroxy 3 metilglutaril CoA reductase) or through the mechanism of increased bile acid synthesis. *Naland (2008)*, the content of niacin in kombucha tea can reduce the excess of cholesterol

in the blood. Vitamin B3 (niacin) has been used to reduce high LDL, cholesterol and triglycerides (fats) blood and increasing HDL effectively.

Conclusion

Kombucha tea fermented in drinking water could decline blood cholesterol level P1 = 9 %, P2 = 13,9 % P3=19,1 %, P4= 12,8 %, respectively. Futhermore for bad cholesterol / LDL decreased P1 = 17,3% P2 = 41,5 % P3 = 55,8 % P4 = 64,4%, and good cholesterol/HDL would rise P1 = 12,93% P2 = 13,73 % P3 = 15,02% P4 = 19,97 % , after consuming kombucha for four weeks.

Uticaj ishrane fermentisanim kombuha čajem na nivoe HDL, LDL i holesterola u krvi pataka

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Rezime

Kombuha sadrži visok nivo glukuronske kiseline, supstance koja vrši neutralizaciju holesterolskih depozita pretvarajući ih u jedinjenja rastvorljiva u vodi. Proučavani su efekti fermentisanog kombuha čaja, kao dodatka u ishrani, na HDL, LDL i ukupan nivo holesterola u krvi pataka. Ogled je obavljen na Fakultetu za stočarstvo-Univerziteta u Padjadjaran-u. Predmet istraživanja bilo je utvrđivanje uticaja različitih koncentracija fermentisanog kombuha čaja na nivoe liporoteina visoke gustine (HDL) i liporoteina niske gustine (LDL) kao i na nivo ukupnog holesterola u krvi pataka. U istraživanju je korišćen eksperimentalni metod sa kompletno slučajnim modelom (CRD). Ogled je obuhvatio pet tretmana (P0 = 0%, P1 = 10%, P2 = 15%, P3 = 20%, and P4 = 25%) i četiri ponavljanja. Rezultati su pokazali da je dopunska ishrana svim tretmanima značajno smanjila nivo LDL i ukupnog holesterola dok je nivo HDL povećan. Može se zaključiti da dodavanje fermentisanog kombuha čaja do 25% u ishrani snižava nivoe LDL i ukupnog holesterola uz povećanje nivoa HDL.

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INFLUENCE OF SPRING FEED ON THE STRENGTH OF HONEY BEE COLONIES DURING SPRING DEVELOPMENT

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Abstract: The strength of honey bee colonies during year depends on wintering and on biologic development of colonies during spring period. To ensure satisfactory colony development in spring period, it is necessary to add stimulative feed. The aim of this study is to determine the effect of different types of spring feed on the honey bee colony strength. Twenty honey bee colonies were selected for this experiment. Colonies were divided into five groups, and each group received different stimulative feed. The first group was fed with sugar syrup, and the second with sugar syrup with added microelements and with vitamin complex. The third group received sugar candy without additives, and the fourth group received sugar candy with addition of microelements and vitamins. The fifth group was fed with honey. The experiment was conducted on the apiary of the Institute for forage crops in Kruševac.

Key words: honey bee, spring development, stimulative feed

Introduction

The main food for honey bees (*Apis mellifera* L.) are pollen and honey. Pollen represents the main source of protein, fat, vitamins and minerals in the honeybee diet. On the other hand, honey is rich in carbohydrates (fructose and glucose).

Spring development is very important aspect of honey bee activity. To reach full potential for honey production, colonies must produce large number of young workers. To accomplish this, it is necessary to add nutrients to stimulate development of young worker bees as many studies show that pollen is necessary for full development of hypopharyngeal glands in young bees. These glands secrete jelly and royal jelly required by honeybee larvae *(Maurizio, 1950; Standifer et al., 1960)*. This indirect relationship of pollen-derived nutrients to larval development

has been demonstrated by many investigators over the past 40 years (*Langer, 1931; Haydak, 1970; Dietz, 1975)*.

Materials and Methods

The experiment was conducted in the apiary of Institute for forage crops in Mačkovac, Kruševac. The selected colonies were of the similar strength and food supply. Twenty honey bee colonies were used for this experiment. Selected colonies were divided into five groups. To each group were given different stimulative feed:

Group 1 – sugar syrup consisting of equal ratio of edible sugar (sucrose) and water,

Group 2 – sugar syrup of same composition as above but with added microelements (Oligovit pills),

Group 3 – sugar candy, prepared by standard recipe for this type of solid honey bee feed,

Group 4 – Sugar candy with the addition of microelements and vitamins (Forsapin),

Group 5 – honey.

Following parameters were studied: the amount of bees, the amount of brood, the amount of honey and the amount of pollen. The studied traits were assessed by standard grade system used in beekeeping.

The experiment lasted from April 2011 to May 2011.

Results and Discussion

All groups showed accelerated development, which is common when the stimulative feed is applied (table 1 and 2).

The first group showed the increase of 96.38% in the amount of bees, 65.51% in the amount of brood, 18.94% for the amount of honey and 44.25% for the amount of pollen.

Group 2 had similar trend, where all studied traits, also, showed increase. Here the increase was as following: 77.47% for the amount of bees, 71.67% for the amount of brood, 17.22% for the amount of honey and 65.42% for the amount of pollen.

In the third group, increase in all values for studied traits was, also, determined. Here the results were as following: 67.59% for the amount of bees, 68.48% for the amount of brood, 6.97% for the amount of honey and 30.08% for the amount of pollen.

As with previous, the fourth group similar results, as all values increased: 71.13% for the amount of bees, 86.29% for the amount of brood, 17.39% for the amount of honey and 51.55% for the amount of pollen.

The values for studied traits in the fifth group also showed increase: 64.02% for the amount of bees, 76.05% for the amount of brood, 16.10% for the amount of honey and 20.98% for the amount of pollen.

Table 1. The average values for studied traits by groups at the beginning of the experiment

Group	Amount of bees	Amount of brood	Amount of honey	Amount of pollen
1	4,70	2,90	2,27	1,13
2	5,37	2,93	2,73	1,07
3	5,03	2,57	3,30	1,23
4	5,30	2,70	2,07	0,97
5	5,67	2,63	2,67	1,43

Table 2. The average values for studied traits by groups at the end of the experiment

Group	Amount of bees	Amount of brood	Amount of honey	Amount of pollen
1	9,23	4,80	2,70	1,63
2	9,53	5,03	3,20	1,77
3	8,43	4,33	3,53	1,60
4	9,07	5,03	2,43	1,47
5	9,30	4,63	3,10	1,73

The highest increase in the amount of honey bees per colony was determined in the first group which was fed only with sugar syrup. The development of the brood was most increased in the group 5 which was fed with honey. The differences in the amount of honey per colony were somewhat similar in all groups (except in the group 3). Higher differences can be seen in the amount of pollen, which can be explained by the differences in foraging urge between colonies.

Conclusion

According to the collected data from this experiment it can be concluded that the stimulative feed in the spring is very important factor in the honey bee colony development. The addition of vitamins and minerals have increased the development of the colonies, but the most developed colonies were ones which received pure honey and sucrose syrup, meaning that these types of honey bee feed contain enough matter for normal colony development.

Uticaj načina prolećne prihrane na snagu pčelinjih društava tokom prolećnog razvoja

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Rezime

Snaga pčelinjih društava u toku godine zavisi od samog prezimljavanja, kao i od biološkog razvoja društava u toku prolećnog perioda. Da bi se osigurao zadovoljavajući razvoj društava u prolećnom periodu, neophodno je vršiti stimulativnu prihranu pčelinjih društava.

Cilj rada je da se utvrdi efekat različitih načina prolećne prihrane na snagu pčelinjih društava.

Za ogled je odabrano 20 pčelinjih društava koja su podeljena u pet grupa i svakoj grupi je dodavana određena stimulativna prihrana. Prva grupa je prihranjivana šećernim sirupom, a druga grupa šećernim sirupom obogaćenim mikroelementima i vitaminskim kompleksom. Treća grupa je dobijala šećerne pogače bez dodataka, dok su četvrtoj grupi dodavane šećerne pogače obogaćene proteinima. Peta grupa je prihranjivana medom. Ogled je izvršen na pčelinjaku Instituta za krmno bilje u Kruševcu.

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CORRELATION OF MORPHOLOGIC AND PRODUCTION TRAITS OF HONEY BEE COLONIES FROM SERBIA

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Original scientific paper

Abstract: State of mutual or reciprocal relationship between traits is determined by the correlation coefficients. Correlation of morphological and production traits was determined in two generations of honey bee colonies. In the first generation, mother queen bees were studied, and in the second generation, their offspring. The study of one generation lasted two years. Morphological traits were determined in the beginning of the study and production traits were determined in the spring and autumn inspections of every year. In the first generation, the positive and mostly high correlation between morphological traits, and honey yield and hygienic behaviour was determined. Correlation of morphological traits, colony strength and food supplies per colony ranged from highly positive to slightly negative values. It was found that there was a high correlation between some production traits (colony strength in the spring and autumn inspections and honey yield per colony). In the analysis of offspring (second generation), most traits that were positively correlated in mother generation now, also, showed a similar relationship. The only difference, in contrast to mother generation, was that, for most traits, the intensity of interaction increased.

Key words: honey bee, correlation coefficient, morphologicl traits, production traits

Introduction

Correlation is interrelationship between the occurrences presented by different values of the two variables. This connection means that the value of variables can be predicted with a certain probability based on knowledge of the values of other variables. Correlation coefficients measure expression of the relationship between two variables in the units independently of the specific units

of measure of variables. Determining the correlation relationship between different traits is especially important in honey bee breeding programs. Particular attention to correlation relationships between different traits in honey bees and was given by *Билаш and Кривцов (1991)*. In sampling of the colonies for further selection, indirect selection methods based on the correlation coefficients are placed just behind the method of large-scale phenotypic selection and methods of genotypic individual selection. They claim that this method is based on the correlative variability, meaning that change in one of traits triggers changes in other traits which are in the correlation relationship with previous trait.

Above mentioned authors claim that the honey yield is in positive correlation with tongue length (r = 0.22 to 0.72), front wing length (r = 0.69), front wing width (r = 0.31), third tergit length (r = 0.63) and the cubital index (r = 0.32). As for the other traits of the honey bee colony, productivity is the most correlated with the number of eggs laid by honey bee queen per day 36 days from the beginning of foraging (r = 0.73) and intensity of bee flights (r = 0.62). They state that the tongue length is negatively correlated with fertility of queen bee and resistance to *Nosema*. *Mladenović and Radoš (2010)* have established the existence of a positive correlation between the colony strength and the amount of honey and pollen in the colony. The highest level of interaction is achieved between the number of bees and the amount pollen in the colony (r = 0.48), while the lowest recorded interaction is between the amount of pollen and the amount of honey (r = 0.13). The honey yield is correlated with the lifespan of worker bees (Milne, 1980), one-day intake of honey *(Szabo, 1981)*, brood area *(Georgiev and Plavša, 2005)*, meteorological factors and the capacity of the honey flora *(Mladenovic, 2006)* and other factors.

The aim of this study was to determine the correlation coefficients of morphological traits (tongue length, length and width of the wing and basitarsus) and production characteristics of honey bee (colony strength and food supply) as determined in the autumn and spring examination with a total yield of honey per company and the expressed hygienic behaviour.

Materials and Methods

In this experiment, 18 colonies were studied. Colonies were sampled from different regions of Serbia (Rasina, Pester, Morava, Banat, Timok and Kopaonik). Morphological traits were determined using binocular, brand "Olympus", with a magnification 25x, which has a measuring eyepiece which was used for the measurement of honey bee body parts. During the spring examination, 50 bees were taken from each colony and suffocated, then their body parts were separated (head, wing and leg), placed on the slide and measured using binocular.

The amount of bees, brood area, honey and pollen in the colony were estimated visually during the spring (the last decade of March) and autumn examinations (the first decade of September). These traits are expressed in taken size per frame (1 / 10) or in percentage. Honey yield was determined as the total honey subtracted from the colony with the honey left for the winter. The amount of honey left for the winter is determined by the area of caped honey on 1dm2 calculated as 0.25 kg and 1dm2 uncapped as 0.15 kg of honey.

Hygienic behaviour was determined using pin killed method.

For morphological traits (tongue length, length and width of tarsus), production characteristics and quantity of collected honey, correlation coefficients were calculated according to formulas by *Hadživuković (1991)*. Correlation coefficients were calculated separately for the parent colonies and for offspring colonies. The correlation coefficients were calculated by the following formula:

$$r = \frac{\sum_{i=1}^{n}(Xi - \overline{X})(Yi - \overline{Y})}{\sum_{i=1}^{n}(Xi - \overline{X})^2 \sum_{i=1}^{n}(Yi - \overline{Y})^2}$$

Following scale was used for the strength of correlation:

0.00 to 0.30 - weak correlation;

0.31 to 0.60 - medium strong correlation;

0.61 to 0.90 - strong correlation;

Standard errors of correlation coefficients were calculated by the formula (Hadživuković, 1991):

$$S_r = \frac{\sqrt{1 - r2}}{\sqrt{n - 2}}$$

Testing of the significance of correlation coefficients was performed by t-test using the following formula and table by Snedecor (Hadživuković, 1991):

$$t = \frac{r}{Sr}$$

for n - 2 freedom degrees from the table of t-distribution, where n is the number of colonies in the experiment.

Results and Discussion

Correlation coefficients of the basic traits in parents. In has been established that in parent colonies there is a strong correlation between the morphological traits and honey yield and between morphological traits themselves, except for tarsal index which is negatively correlated with all morphological traits (Table 1). The amount of bees in the spring inspection was in strong correlation with brood area and pollen in both, spring and autumn, inspections, the honey yield and the number of foragers before and after noon. The amount of bees in the spring was in the medium-high correlation with the amount of bees in the autumn inspection. The amount of bees in the spring inspection was in strong correlation with brood area in the autumn and the honey yield. Brood area in the autumn was in strong correlation with the honey area in the spring of and honey yield, and in medium strong correlation with the honey area and pollen in autumn. Honey area in the autumn was in strong correlation with the honey yield per colony. Other traits were mainly in the medium strong correlative relationships. In parent colonies, honey yield was in strong and medium strong correlative relationships with most of the observed traits. From the observed traits, amount of honey in spring and autumn inspections showed the least correlation with other observed traits. Manifested hygienic behavior was determined in 24 and 48 hours after damaging the cells, and it showed a strong correlation with the wing width ($r = 0.61$ and $r = 0.53$) and medium-strong correlation with the tongue length ($r = 0.38$). It was determined that colonies that exhibit hygienic behavior in the first 24 hours after the damage, also do it after 48 hours ($r = 0.72$). Hygienic behavior was in a weak or negative correlation with any other production traits

Table 1 The correlation coefficients for the productive traits of the maternal colonies

	Wing length	Wing width	Basitarsus length	Basitarsus width	Tarsal index	Amount of bees in spring	Amount of bees in autumn	Brood area in spring	Brood area in autumn	Honey in spring	Honey in autumn	Pollen in spring	Pollen in autumn	Honey yield	Hygienic behavior	
															24 h	48 h
Tongue length	0,11	0,53	0,55	0,46	-0,52	0,04	0,13	-0,07	0,02	-0,27	-0,19	-0,12	-0,18	0,46	0,38	0,38
Wing length		-0,22	0,36	0,54	-0,08	-0,12	0,07	-0,23	0,15	0,13	0,22	0,21	0,10	-0,01	-0,09	-0,03
Wing width			0,35	0,20	0,03	-0,06	0,05	-0,16	0,05	0,20	-0,07	-0,23	-0,28	0,13	0,53	0,61
Basitarsus length				0,73	-0,23	0,11	0,60	0,07	0,39	-0,01	0,23	0,10	0,17	0,46	0,05	0,26
Basitarsus width					-0,25	0,02	0,43	-0,12	0,44	0,03	0,28	0,25	0,15	0,34	0,18	0,27
Tarsal index						-0,10	-0,08	0,09	-0,04	0,30	0,48	-0,16	0,08	0,15	-0,32	-0,19
Amount of bees in spring							0,51	0,81	0,42	0,21	0,30	0,70	0,69	0,64	0,05	0,33
Amount of bees in autumn								0,57	0,82	-0,14	0,29	0,53	0,57	0,73	0,15	0,07
Brood in spring									0,39	-0,08	0,13	0,47	0,72	0,62	-0,06	-0,28
Brood in autumn										-0,02	0,37	0,66	0,60	0,66	0,24	0,20
Honey in spring											0,57	0,22	0,15	0,21	-0,08	-0,25
Honey in autumn												0,44	0,46	0,60	-0,20	-0,20
Pollen in spring													0,80	0,51	0,09	-0,33
Pollen in autumn														0,72	-0,13	-0,34
Honey yield															0,06	0,04
Hygienic behavior (24 h)																0,72

Correlation coefficients of basic traits in offspring. When testing offspring (queen bee daughters) in the third and fourth year, most traits which were positively correlated in their parents also showed positive correlation. The only difference when compared to the parents is that for most correlated traits, the intensity of mutual influence (correlation coefficient) was increased (Table 2). Morphological traits were in the medium heavy and strong correlation with each other, except tarsal index, which had a negative correlated to the other morphological traits. Colony strength (the amount of bees and the brood area) was in medium-high and a strong correlation with the honey yield per colony. The highest correlation (r = 0.91) was determined for the amount of bees and the brood area in the spring. Food supply (quantity of honey and pollen) showed minimum correlation with the majority of traits (low or negative correlation). Hygienic behavior of colonies also had very low correlation with majority of traits (mostly negative). The exception was the count after 24 hours and after 48 hours where there was a strong correlation (r = 0.72), which is quite understandable. Hygienic behavior and wing width had medium-strong or strong correlation (r = 0.58 and r = 0.64) which is very interesting. *Bilaš and Krivcov (1991)* state that the tongue length in the Caucasian race of honey bee was positively correlated with the honey yield, however, the same research found that the tongue length was negatively correlated with fertility of queen bees and resistance to the *Nosema*. In addition to the tongue length foraging was highly correlated to the length of the front wing, the third tergit length, maximum number of eggs laid by queen bee, flight intensity, etc. In this source several authors were also cited *(Gold, 1937; Miller, 1939; Bessonet, 1945; Bichtler, 1951; Hunkeller, 1953)* who claim that the honey yield is not in correlation with morphological traits of honey bees. The two-year research by *Plavša and Georgiev (2005)*, it was proved that there was a positive correlation between the amount of bees and brood and the productivity of colonies or the honey yield. *Lebedev (2001)* announces that the input of pollen was in high correlative dependence (r = 0.82) to the colony strength and the amount of brood per colony. *Georgiev et al. (2003)* found that the correlation coefficient between the amount of brood and the amount of pollen in the spring was very high while in the fall it was much lower.

Table 2 The correlation coefficients for the productive traits of the offspring colonies

	Wing length	Wing width	Basitarsus length	Basitarsus width	Tarsal index	Amount of bees in spring	Amount of bees in autumn	Brood area in spring	Brood area in autumn	Honey in spring	Honey in autumn	Pollen in spring	Pollen in autumn	Honey yield	Hygienic behavior	
															24 h	24 h
Tongue length	0,17	0,55	0,65	0,34	-0,39	0,24	0,18	-0,11	0,32	-0,17	-0,08	0,12	-0,09	0,52	0,36	0,39
Wing length		-0,35	0,28	0,61	-0,11	-0,12	0,19	-0,26	0,05	0,11	0,28	0,24	0,03	0,01	-0,09	-0,23
Wing width			0,38	0,22	0,23	-0,16	0,12	-0,13	0,19	0,23	-0,01	-0,28	-0,35	0,26	0,58	0,64
Basitarsus length				0,71	-0,24	0,16	0,62	0,13	0,42	-0,11	0,32	0,15	0,29	0,46	0,01	0,28
Basitarsus width					-0,25	0,11	0,45	-0,04	0,32	0,03	0,29	0,34	0,17	0,38	0,21	0,22
Tarsal index						0,00	-0,02	0,02	-0,06	0,24	0,32	-0,12	0,08	0,11	-0,22	-0,16
Amount of bees in spring							0,52	0,91	0,25	0,03	-0,21	0,26	0,24	0,61	0,14	0,34
Amount of bees in autumn								0,51	0,24	0,19	0,28	0,01	0,10	0,71	-0,10	0,02
Brood in spring									0,45	0,04	-0,18	0,39	0,33	0,55	0,13	0,30
Brood in autumn										0,20	0,25	0,15	0,69	0,37	0,10	0,07
Honey in spring											0,59	-0,30	0,05	0,16	0,11	0,05
Honey in autumn												-0,23	0,12	0,32	-0,04	-0,23
Pollen in spring													0,17	0,18	0,34	0,11
Pollen in autumn														0,29	0,58	0,49
Honey yield															-0,07	0,09
Hygienic behavior (24 h)																0,83

Conclusion

During the four-year study of two generations of honey bee colonies it was determined that some morphological and some production traits have a major impact on the overall productivity of colony or the honey yield of.

Most morphological traits (except tarsal index) showed a medium strong and strong correlation to each other and to the productivity of colony.

Colony strength (the amount of bees and the brood area) in both generations exhibited strong and medium strong correlation with the honey yield. Weaker correlation was determined between food supply (amount of honey and pollen) with the honey yield of honey, and this is particularly evident in the second generation.

Expression of hygienic behaviour in the colony had a low correlation to the majority of traits. Only the wing width ($r = 0.58$ and $r = 0.64$) and tongue length ($r = 0.36$ and $r = 0.39$) had showed strong or medium-strong correlation with this trait

Korelacija morfoloških i proizvodnih osobina društava medonosne pčele sa područja Srbije

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Rezime

Stanje uzajamne ili recipročne povezanosti jedne osobine od druge utvrđuje se na osnovu koeficijenta korelacije. Korelacija morfoloških i proizvodnih osobina utvrđivana je kroz dve generacije društava medonosne pčele. U prvoj generaciji praćene su majke matice, a u drugoj njihovo potomstvo. Ispitivanje po jednoj generaciji trajalo je dve godine. Morfološke osobine su utvrđene na početku praćenja generacija, a proizvodne na prolećnom i jesenjem pregledu svake godine.

U prvoj generaciji utvrđena je pozitivna i mahom visoka korelacija između morfoloških osobina, prinosa meda i higijenskog ponašanja. Stepen korelacije morfoloških osobina, snage društva i zaliha hrane po društvu bio je od visoko pozitivne do slabo negativne vrednosti. Ustanovljeno je da postoji i visok stepen korelacije između pojedinih proizvodnih osobina (snaga društava na prolećnom i jesenjem pregledu i prinosa meda po društvu).

Prilikom testiranja potomstva (II generacija) većina osobina koje su bile u pozitivnoj korelaciji kod roditelja i sada su pokazivala uzajamni odnos. Jedina razlika u odnosu na roditelje je u tome da se, za većinu osobina, intezitet međusobnog uticaja povećao.

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THE AUTHENCITY OF HONEY IN RELATION TO QUALITY PARAMETERS

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In the Serbian market can be found significant quantity of honey which authenticity can be doubt. Honey is changed sensory, as well as in certain chemical quality parameters. Sometimes it happens that honey is in accordance to quality requirements but that doesn't mean that it's natural and authentic honey. Many analysts who are engage in testing the quality of honey have experience to recognize the honey that isn`t from honey bees but the work of skilled producers. The question is how to solve the problem - how to recognize forgery honey from natural although it is in accordance to quality requirements prescribed by the legislation? The most important is that this honey isn`t selling to consumers as a natural honey. Analysts here are becoming helpless because the law is rigid and based on the quality parameters that we cannot with certainty tell if the honey is forgery or not. In this article we reviewed the results of examination of quality of honey from the market of Serbia, where the sensory evaluation demonstrated that it is a honey in which authenticity can be doubted. The results were: 1. honey that has sensory changed with good quality parameters and 2. honey that has sensory changed with quality parameters which isn't in accordance with Book of Regulation. The conclusion is that a larger number of samples that have been sensory changed did not satisfy the chemical quality parameters, and in one part of the samples were present residues of antibiotics and sulfonamides.

Key words: honey, authenticity, chemical quality parameters, sensory evaluation

Introduction

Honey is a food of animal origin and in our region is a product of the honey bee - Apis meliffera carnica. Honey is a unique food that contains all the necessary elements for growth and development of organisms (amino acids, carbohydrates, vitamins, organic acids, minerals, pollen, essential oils, proteins, enzymes, etc.). The quality of honey depends on the geographical origin and presence of the plant species on the site. In Serbia, the most common are acacia and meadows - flower honey, and lime and sunflower honey. Other types of honey

appear in small numbers. Honey is used by all groups, but it is recommended to children, athletes, convalescent and elderly people because of the rich content of vitamins, minerals, enzymes, amino acids and others. The crisis in society leads to a crisis of morality, so in the market can be find a certain amount of honey that are not authentic - natural *(Dugalić-Vrndić et al., 2005, 2006)*. Based on the Book of Regulations for quality and other requirements for honey, bee products, honey based preparations and other bee products *(Official Gazette of Serbia and Monte Negro, no. 45/03)*, honey has to have intrinsic color, aroma and taste, and may be liquid or pureed consistency, partially or completely crystallized. Sensory analysis is a science method that measures and evaluates the characteristics of the product (color - appearance, consistency, odor and taste) with one or more senses and as such is one of the most important parameters in defining the overall quality of honey *(Popov-Raljić et al., 2005, 2006, 2007)*. Based on sensory analysis, a number of honey samples did not in accordance to the Book of Regulations. Therefore, we can doubt the authenticity of these samples. However, these samples of honey are impossible to declare a forgery on the basis of quality parameters prescribed by legislation. Some natural components of honey can be find on the market in the synthetic form (e.g. diastase, HMF) and when examining the standard methods cannot be determined the origin of these components, and this creates a problem to analysts recognize forgeries, and it cannot prove the prescribed test methods. On the other hand, the use of antibiotics and sulfonamides in the treatment of bees that use unskilled persons leads to the presence of residues of antibiotics and sulfonamides in honey. The aim of this paper is to highlight the problem that is increasingly present in the Serbian beekeeping and that is the appearance of honey that is not natural but on the market is declared as natural and authentic. Such honey is not a safe food for consumption. In addition, the authors propose that the legislation have to include some other quality parameters that would prevent counterfeiting and to thereby bring order to the honey market and protect human health and the honey produced by beekeepers conscientious.

Materials and Methods

Sixty five honey samples were collected and examined in this study. Honey used in this study came from three sources:

1. Honey purchased in supermarkets (25 samples) of different owners, different types of honey (mountain, meadow, flower, forest, etc.)
2. Honey samples (25 samples) taken from the famous beekeepers on–site production
3. Honey from the laboratory of the Institute, which is encrypted arrived for examination (15 visually altered samples).

All of honey samples were placed in jars made of glass or plastic. In all the samples the first was carried out sensory evaluation. Color - appearance, odor, taste and consistency were tested by sensory evaluation without use any operating tools.

Color - appearance of honey was examined in the following way: approximately 40 g of honey were measured in a transparent glass balloon (160 ml) at a temperature of 18^0C to 25^0C and colors and hue were evaluated. Determination of odor - olfactory technique: about 40 g of sample was measured in a glass balloon of 160 ml at room temperature. Honey sample is taken with plastic spoon and placed in front of nose and intensity of flavor and aroma are estimated by inhaling volatile compounds. Sensory evaluation of taste: 1-2 g of honey is weighed at room temperature then tasted and evaluated. Consistency/texture of honey is determined based on mechanical, geometric and surface properties which can be observed mechanical, tactile and where is possible, with visual and auditive receptor.

After sensory evaluation six quality parameters were determinate: reducing sugars, sucrose (HPLC/RI), moisture (refractometry), acidity (volumetry), diastase activity (UV/Vis spectrophotometry) and HMF (HPLC/UV) were determined using standard methods of the International Honey Commission (IHC) and the presence of residues of antibiotics and sulfonamides. Examination of residues of antibiotics and sulfonamides was done in parallel using two microbiological methods - Premi® test and Four plate methods.

Results and Discussion

Based on the sensory evaluations of 65 honey samples with the methods used human senses (visual, olfactory, tactile and auditory receptors) (*Popovic-Raljić et al., 2005, 2006, 2007.*) Results are obtained, where 40 (61,5 %) of honey samples does not correspond to sensory specifications given in ISO standard. Table 1 and 2 shows the results of sensory evaluation.

Table 1. Sensory evaluation of honey quality (source *Popović-Raljic et al. 2005* modified by the authors)

Honey origin	Number of samples	Description of the sensory characteristics			
		Color - appearance	Odor	Taste	Consistency
Source 1	25	inconsistent, inappropriate	inappropriate, unpleasant, atypical note	inappropriate	liquid, viscous and Granulated in 2 - 3 layers
Source 2	25	color intensity, hue, saturation and purity consistent and appropriate	odor intensity highly strong that can be identified	highly inherent basic taste that lasts longer than 5 minutes	creamy inherent (fine crystals)
Source 3	15	inconsistent, inappropriate	inappropriate, unpleasant, atypical note	inappropriate	liquid, viscous and Granulated in 2 - 3 layers

Table 2. Results of sensory evaluation

Honey origin	Number of samples	Results of sensory evaluation		
		In acc.*	Not in acc.**	Evaluation
Source 1	25	-	25	0
Source 2	25	25	-	3
Source 3	15	-	15	0
Total %	65 100 %	25 38.5 %	40 61.5 %	

Table 3. Results of laboratory examination of honey samples I

Honey origin/ Parameters	Source 1		Source 2		Source 3	
	In acc. *	Not in acc. **	In acc. *	Not in acc. **	In acc. *	Not in acc. **
Reducing sugars MRL min. 60 %		+ (6/25)	+ (25/25)			+ (1/15)
Sucrose MRL max. 5 %	+ (25/25)		+ (25/25)		+ (15/15)	
Moisture MRL max. 20 %	+ (25/25)		+ (25/25)		+ (15/15)	
Acidity MRL max. 40 mmol/kg	+ (25/25)		+ (25/25)		+ (15/15)	
Diastase activity MRL min. 8 DN		+ (4/25)	+ (25/25)			+ (4/15)
HMF MRL max. 40 mg/kg		+ (8/25)	+ (25/25)			+ (5/15)
Residues of antibiotics and sulfonamides		+ (5/25)		+ (3/25)		

Table 4. Results of laboratory examination of honey samples II

Honey origin	Number of samples	Quality (chemical parameters)		Residues of antibiotics and sulfonamides	
		In acc.*	Not in acc.**	Present	Not present
Source 1	25	7	18	5	20
Source 2	25	25	-	3	22
Source 3	15	5	10	-	15
Total %	65 100 %	37 56.9 %	28 43.1 %	8 12.3 %	57 87.7 %

*in accordance with the Book of Regulations/per samples
**not in accordance with the Book of Regulations/per samples

Based on the results of testing of 65 honey samples, 40 samples are not in accordance to the sensory evaluation, 28 samples are not in accordance to chemical parameters of quality and in 8 samples we found the presence of residues of antibiotics and sulfonamides (tables 2 and 4). Probably it might be higher presence of residues of antibiotics and sulfonamides probably if we had more authentic honey samples than the honey of doubtful origin.

Source 1: according to sensory evaluation of 25 honey samples taken from the market, it was found to be inadequate due to changed sensory characteristics (appearance, taste, odor, color and consistency). In the same group (source) 18 samples of honey were not in accordance with the legislation, while the other 7 were in accordance. The presence of antibiotics and sulfonamides was found in 5 samples. The results are similar to research *Dugalić-Vrndić et al. (2005, 2006).* where they established the presence of residues of antibiotics and sulfonamides in honey purchased in supermarkets in the low percentage of the total number of examined samples (2 %). Uncontrolled use of antibiotics has resulted in their retaining in honey and other bee products, and studies have found that oxytetracycline retains after 40 to 46 days in the weak and medium bees colonies, while in the strong bee colonies they decompose after 25 days. *(Plavsa et al., 2005*.). Compared to other countries in the EU, where the presence of antibiotics have been found in up to one third of honey that are on the market *(Bogdanov et al, 2007)*, honey purchased in supermarkets in Serbia is much less contaminated, as can be seen from the results of this research

Source 2: honey sampled on the place of production of known beekeepers, without notice, gave good results in tests of sensory evaluation and chemical parameters, however in a certain number of honey we found the presence of residues of antibiotics and sulfonamides (3 honey samples). Results are similar to the results of *Dugalić-Vrndić et al., 2005*, they are found residues of antibiotics and sulfonamides in 18% of examined samples. Similar results related to the chemical quality parameters gave research *Matović et al. (2009),* where 11.48 % of honey samples found to not in compliance with legislation.

Source 3: Honey visually changed, arrived at the Institute for examination (15 samples). All 15 samples were not suitable sensory (appearance, taste, odour, color and consistency), in 5 samples were not found HMF (hydroxymethyl furfural) and these samples are the exception in laboratory practice. Hydroxymethyl furfural is the cyclic aldehyde resulting from dehydration of fructose and glucose in acidic conditions *(Tosi et al., 2004; Dugalić-Vrndić et al., 2010)* and continues to be broken down into formic acid and levulinic acid. Speed of decomposition is higher at elevated temperatures and increase in speed of decomposition is proportional to the temperature rise. In between HMF occurs naturally, its content is low in fresh honey and ranges from 0.6 to 2 mg/kg. Honey that was examined came in the winter when the honey had the lowest age and in such of samples HMF expected to attend in a certain amount. The remaining 10 samples with altered sensory and

visual properties had satisfactory quality parameters. Here is a problem: How to honey in which authencity we suspect declare as a forgery based only on sensory analysis and quality parameters which are in accordance to Book of Regulation? Based on analysis scientists know that this honey is not natural, but what to do when the parameters prescribed by the Book of Regulations have a value within the allowable limits? No doubt legislation must be applied, but this gives the possibility to forged honey gets on the market as genuine - natural honey.

Conclusion

Based on the research can be concluded:

- 61.5% of honey samples have been changed sensory
- With 43.1% of values honey samples of chemical quality parameters (HMF, reducing sugars, diastase) were not in accordance with legislation prescribed MRL
- 12.3 % of samples were contaminated with antibiotics and sulfonamides

Comparing the percentages of samples that did not in accordance tosensory and quality regulations, the number of samples that had been changed sensory greater than the number that did not correspond to chemical quality parameters although it was expected that this number be equal.

On the basis of the research and scientific and professional knowledge, the authors recommend the implementation of new methods, i.e. quality parameters: pollen and isotopic analysis of honey (testing the authenticity and geographical origin). Honey pollen analysis is a method, supported by chemical analysis, which allows identification of forged honey. Pollen grains in honey are a significant indicator of origin of honey - pollen of plant species is a fingerprint and in addition can also be determined the geographical origin of honey. While measurement of stable isotopic composition allows evaluation of food adulteration of natural sweeteners with high fructose corn syrup.

Artificial honey and a presence of residues of antibiotics and sulfonamides is becoming a problem in Serbia and in its resolving must be team-work (the authorities of the legal and criminal policy, professional and scientific workers in finding research methodology) in order to protect human health and production of high quality foods such as honey.

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Rezime

Na tržištu u Srbiji se može naći veća količina meda u čiju se autentičnost sumnja. Med je promenjen senzorno, kao i u određenim hemijskim parametrima kvaliteta. Dešava se da med ispunjava uslove kvaliteta, a da ipak nije prirodan i autentičan. Mnogi analitičari koji se bave ispitivanjem kvaliteta imaju iskustva da prepoznaju med koji ne potiče od medonosnih pčela, već je delo umešnih proizvođača falsifikovanog meda. Tu nastaje problem – kako med koji jeste falsifikat a ispunjava sve uslove kvaliteta propisane zakonskom regulativom proglasiti falsifikatom? Od primarnog je značaja da se takav med ne dospe na tržište kao prirodan med. Analitičari tu postaju bespomoćni jer je zakon rigidan i na osnovu parametara kvaliteta koji su njim propisani ne može se sa sigurnošću tvrditi da je med falsifikat ili ne. U ovom radu dat je pregled rezultata ispitivanja kvaliteta jednog broja uzoraka meda sa tržišta Republike Srbije, gde su senzorne ocene pokazale da je u pitanju med u čiju se autentičnost može sumnjati. Dobijeni su sledeći rezultati: 1. med koji je senzorno promenjen sa dobrim parametrima kvaliteta i 2. med koji je senzorno promenjen sa parametrima kvaliteta koji ne odgovaraju Pravilniku. Zaključak je da je kod većeg broja uzoraka med koji je senzorno promenjen nije zadovoljavao hemijske parametre kvaliteta, a kod jednog dela uzoraka koji nisu ispunjavali zahteve propisane zakonom su bile prisutne i rezidue antibiotika i sulfonamida.

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GENETIC TRENDS USING DIFFERENT CRITERIA OF SELECTION ON SPECIALIZED BREEDS IN PIGS

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Original scientific paper

Abstract: The trial had included 8 generations of pigs and last 10 years of has been done at 4 different breeds: Landrace and Yorkshire as dam line and Duroc and Pietrain as a sire line. Trial included 62 sires, 1794 dams, 5694 progeny and slaughtered 838 heads in total. To examine fixed and random effects LS MME model has been used. Installed different selection criteria for L, Y and D, P showed positive and expected improvement. There are know significant differences at age of slaughter between L,Y and D, P showed significantly lower gain and logger age. Genetic trends for certain traits for all used traits showed a positive line. At the same time explain significance of optimal selection criteria during production.

Key words: genetic trends, pigs, litter size, meat content, age.

Introduction

In pig production, from economic point of view, the most important traits can be as follows: number of weaned piglets per sow per year, feed conversion, growth and meat content in carcass. To provide optimal selection effect for each of them, it is important to determine a genetic correlation between them and heritability level as well. According to knowledge of negative genetic correlations between fertility or milk yield and meat content in carcass it is necessarily to develop different selection criteria or better says specialized breeds.

According to a purpose of selection effects on farm production in the analysis we separate due to selection criteria two groups of breed, e.g.: fertility and milking breeds – Landrace and Yorkshire and terminal breeds, e.g.: Duroc and Pietrain. Following literature sources we can aspect some trend which can depend from selection criteria, farm, year and season effect management on the farm too.

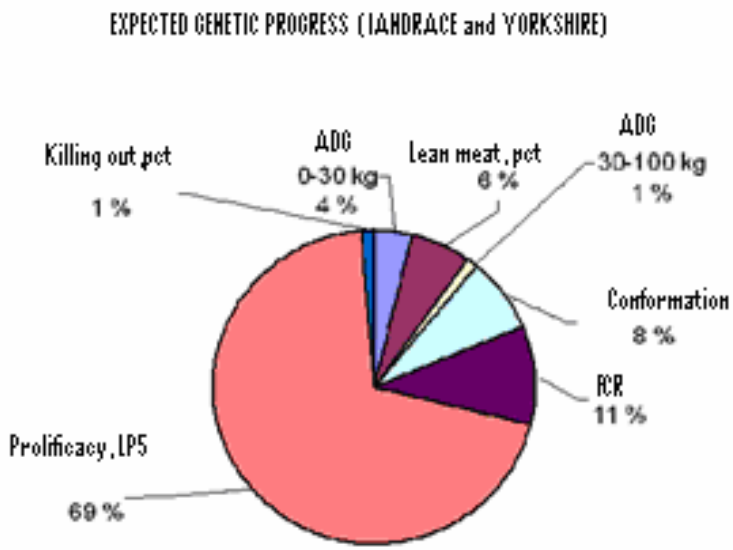
The purpose of research was to analyzed selection effects during more years of selection on farms. Selection criteria were different for terminal breeds (Duroc and Pietrain) compare Landrace and Yorkshire where selection has been concentrated on litter size.

Materials and Methods

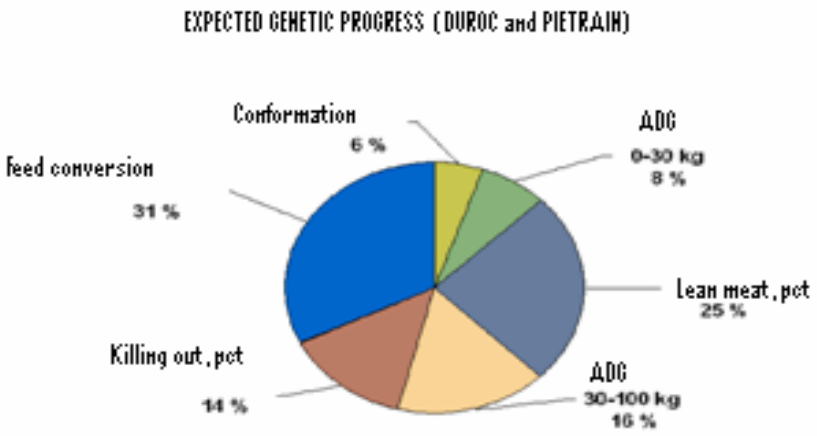
The analysis has been done at 4 farms since 2000 up to 2010. We included 4 breeds, L, Y as mother line and D and P terminal one. The following pictures give different selection criteria that were used. Average live weight at slaughter of all animals was 103 kg.

Table 1: Breed structure and number of animals in trial

Breed No. Carcasses	Sire	Dam	Progeny
Landrace 212	16	714	2.216
Yorkshire 214	18	704	2.164
Duroc 214	16	208	768
Pietrain 198	12	168	546



Graph 1: Used selection criteria for mother breeds: - Landrace and Yorkshire



Graph 2: Selection criteria used for terminal breeds: – Duroc and Pietrain

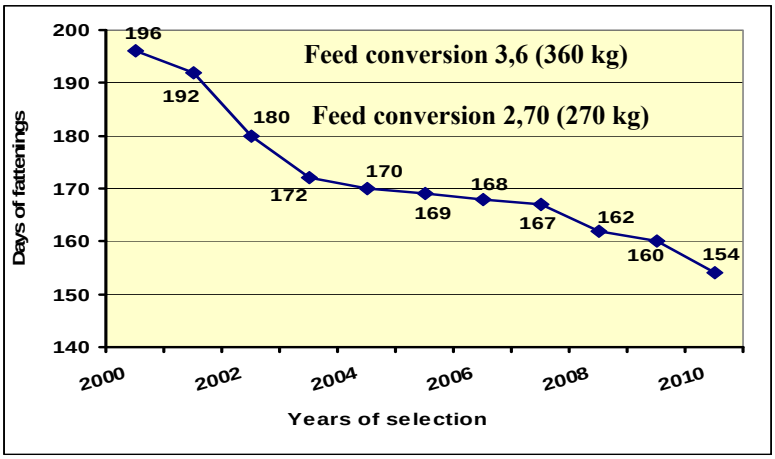
Following MME LS model has been used to analyze influences of FYS (Farm, Year and Season) then breed as fixed effect and Sire as random one.

$$\mathbf{Y_{ijkl} = U + HYS_i + B_{ij} + S_{ijk} + E_{ijkl}}$$

Y_{ijkl} - Number of observations hierarchically distributed;
U - General mean of observations;
HYS_i - Fixed effect of farm, year and season;
B_{ij} - Fixed effect of different breeds;
S_{ijk} - Random sire effect;
E_{ijkl} - Residual

Results and Discussion

Fattening days and feed conversion. Following graphs clearly showed expected tendency. It means we had well defined selection criteria for most economically traits. Intensity of selection has been controlled first of all by a number of dose as per jump. In case of feed conversion (FC) improvements were 90 kg per head. The most fastest improvement was at first 5 years. Since that FC is the average heritage, there are new possibilities to continue with selection effect, e.g. to decrease FC, reduce a cost and increase profit per kg of gain (graph 3). Similar trend has been shown by *Brascamp (1985)*, *Rotschild (1990)*, *Park et al. (1986)* and *Bergsma et al. (2010)*.



Graph 3: Effect of selection on age at sloughter (indirectly on growth) and feed conversion for Landrace, Yorkshire and Duroc.

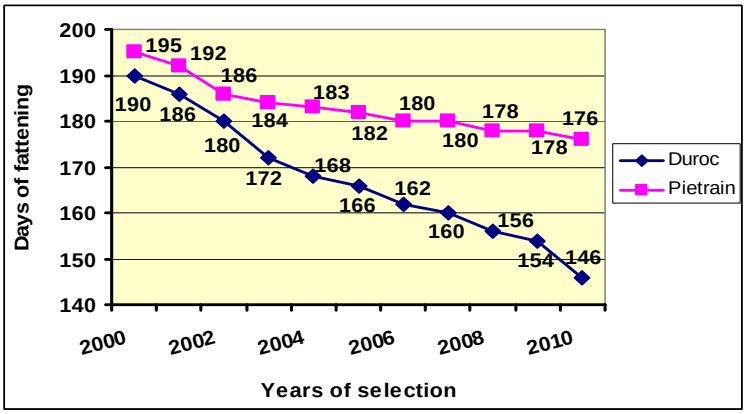
Effects:

1. Less of feed: 90 kg
2. Less fattening days : 42

Notes: There are no selection differences between Landrace, Yorkshire and Duroc. Pietrain had significantly less daily gain and age at slaughter compare to three other breeds.

According to a fattening period, we can recognize improvement of 42 days. Economically it is improvement of about 20 euros per pig.

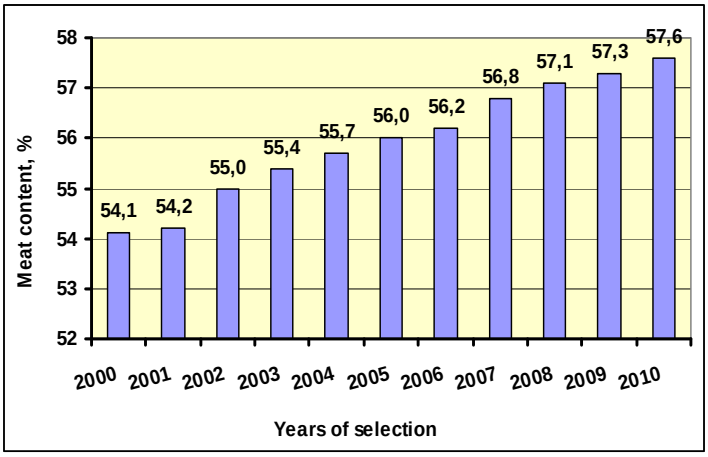
Comparison of age and FC between Duroc and Pietrain differences are present. Duroc had 32 days shorter periods to the certain commercial weight and used 88 kg less food. Feed costs in Duroc are 19 euros less.(Graph 4). These result are similar to *Vidović and Šubara (2010)*, *Vidović et al. (2011)*.



Graph 4: The differences in feed use and age at slaughter between Duroc and Pietrain in test production

The differences: Age: 32 days
Feed: 88 kg

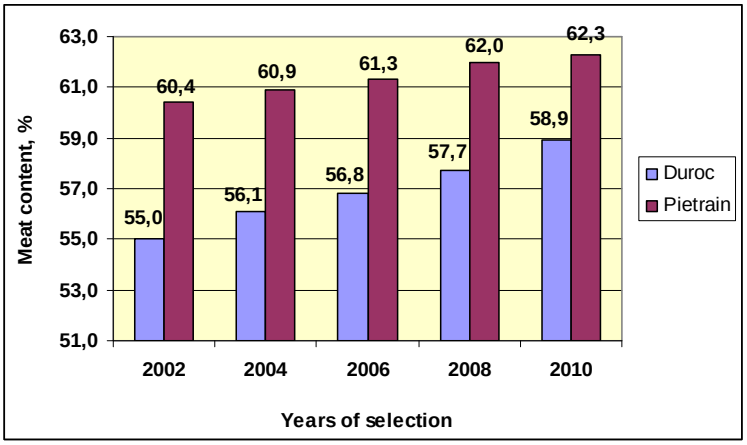
Carcass quality. Meat content in Landrace and Yorkshire has no statistical differences even they showed an optimal trend. Since those to breed are treated as a dam line to provide heterosis effect at F1 daughter's selection criteria were concentrate more on fertility traits. Expected trend were very close to realize one. It is going to back fat between 16- 20 mm at that age. Later these animals if they are going to be parents mast has some reserve to produce progeny (Graph 5).



Graph 5: The trend of selection for percentage of meat content for Landrace and Yorkshire

Just to remind case of present negative genetic correlations between milk yield and meat content in a carcass mean that breeders have to optimize selection criteria and use specialized sire and dam lines in breeding program. In our experiment, we divided into two different groups: L and Y as a mother line and used totally different selection criteria compare to terminal sire lines: D and P. After 10 years of a selection or 8 generations results were present (Graph 5 and 6).

In Graph. 6 we recognized the differences between Duroc and Pietrain even they have the same selection criteria. The only differences were at the beginning of start trial. In conclusion, we can say the trend were more or les the same as selection effect. The differences of 3,4% of meat content or raptly 2,5 kg meat between them provide about 10 euros more profit in fewer to P. But in total D made about 9 euros more profit, including FC as well. Similar conclusions were defined by *Gama et al. (1990)*, *Rotschild (2010)*, *Bergsma et al. (2010)*. Furthermore, analyzes showed both breeds had genetic improvement, faster in D then P.

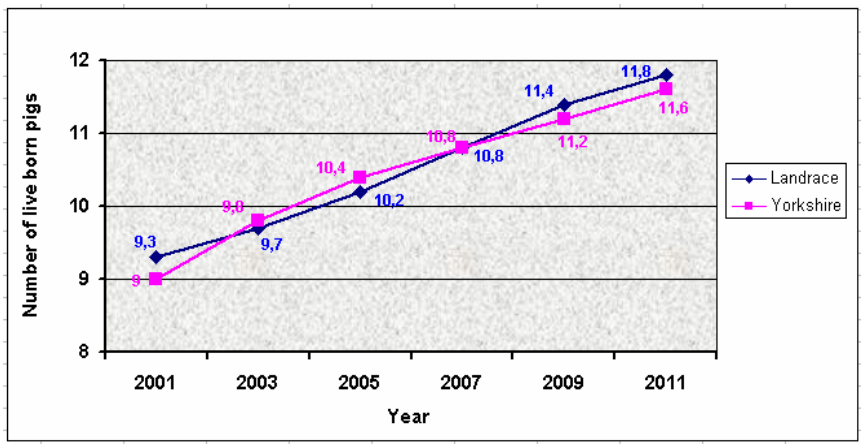


Graph 6: The effects of selection for meat content of Duroc and Pietrain

The effects in meat content, %:

- Duroc: 3,9
- Pietrain: 1,9

Litter size. First of all we used totally different selection criteria of selection in specialized breeds. Litter size was not of selection interest for D and P. So we analyzed trend for L and Y. After generations of selection, selection trend were little less than expected. Probably one of limited factors was FYS effect. Even that selection effect tendency was positive and similar for both L and Y. Similar results have been done by *Nielsen (1994)* and *Vidović at al. (2011)*.



Graph 7: The effect of selection on number of live born piglets in the first farrowing Landrace and Yorkshire.

Conclusion

Genetic trend showed expected tendency for all certain traits. It means selection criteria for certain traits have been optimal.

There have been no selection differences for gain and age at slaughtered Y, L and D. Pietrain showed significantly les gain and needed longer period to reach a certain weight. It means more cost and less profit in case of P.

Selection on meat content had the positive trend. Selection efficiencies were higher at D compare with P. Pietrain itself still have 3,4% more meat in the carcass but much longer period of fattening. Intramuscular fat was much lover at P. This has a negative effect on meat quality.

Litter size showed the positive effect. There are no significant differences between L and Y. Regime of sow feeding showed significant effect concern previous one.

Genetski trendovi korišćenjem različitih kriterijuma selekcije kod specijalizovanih rasa svinja

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Rezime

Desetogodišnja istraživanja izvedena su na 4 farme, obuhvatila su 4 rase (dve plodne – landras i jorkšir te dve terminalne – durok i peitren. Obuhvaćena su

ekonomski najvažnija svojstva: Starost pri kraju tova, indirektno prirast; Sadržaj mesa u polutkama te povećanje veličine legla na zalučenju, indirektno povećanje broja živorođenih u leglu i smanjenje broja praznih dana. Istraživanja su sprovedena na 62 oca, 1.794 majke, 5.694 potomka od kojih je disekcirano 838 grla. Za korekciju fiksnih i slučajnih uticaja na genom je primenjen mešoviti model metoda najmanjih kvadrata.

Selekcijski kriterijumi za oba tipa ispitivanih grla (plodne rase: landras i jorkšir te durok i pietren) prikazan je u radu i odnosi se na očekivanje promene tokom generacija selekcije. Dobijeni rezultati potvrdili su postavljenu hipotezu o mogućem selekcijskom trendu. Tehnologija hranjenja krmača bila je istovetna za oba genotipa plodnih i terminalnih rasa.

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SELECTION EFFICIENCY ON BONES AND MEAT YEALD IN PIGS

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Original scientific paper

Abstract: Trial were conducted at 5 farms, on 5 breeds of pigs in itch farm, in a period from 2000 to 2010. To analyzed data, the MME LS model was used to correct FYS and Breed as fixed effect and Sire as random one. Genetic parameters were estimated from half siblings group. Bones had significantly smaller weight in Pietrain pigs compared to all other breeds. Furthermore, daily and life gains at the Pietrain breed were significantly smaller compared to other breeds. Heritability estimates were significantly different for three breeds, higher for bones, higher for meat yield. The pheneotipic and genetic variations were similar for all traits. Economic effect of selection can be analyzed and expected with a positive trend. Genetic correlation showed expected trend. It is possible to use the effect of indirect selection as well.

Key words: pig breeds, selection, bone, meat.

Introduction

Feed conversion, meat yield and litter size are most important traits in pig production. The capacity of animal is a high genetic correlation with meat yield, gain, litter size and profit.

To provide selection effect and improve the value for each trait included in aggregate genotype it is necessary to use economic value for each one at the same time with genetic parameters together with intensity of the selection and generation interval. Breed structure and selection criteria are valuable for long time run of breeding program.

The purpose of this research was to optimize and provide selection criteria and selction effects for specialized breeds using ferther for crossbreeding in commercial farms production.

Materials and Methods

The analysis has been done at 5 farms since 2000 up to 2010. We included 5 breeds, L, Y as mother line and D, H and P terminal one. The different selection criteria have been installed, same for L and Y and as a dam line and same for sire line, D, H and P *(Vidović et al., 2011)*. Average live weight at slaughter of all animals was 102 kg. The variation statistics have been used to estimate variability parameters. MME LS model has been used to analyze influences of FYS (Farm, Year and Season) then breed as fixed effect and Sire as random one. Genetic parameters, e.g. heritability and genetic correlations were estimated from half sibs group.

$$Y_{ijkl} = U + HYS_i + B_{ij} + S_{ijk} + E_{ijkl}$$

Y_{ijkl} - Number of observations hierarchically distributed;
U - General mean of observations;
HYS_i - Fixed effect of farm, year and season;
B_{ij} - Fixed effect of different breeds;
S_{ijk} - Random sire effect;
E_{ijkl} - Residual

Structure of breed and category of animals are showed in Table 1.

Table 1: Breed structure and number of selected animals

Breed	Sire	Dam	Progeny No. Carcasses
Landrace	21	814	2.326 234
Yorkshire	20	824	2.336 242
Duroc	17	238	769 222
Pietrain	12	172	522 201
Hampshire 56	8	88	68

Results and Discussion

All results are presented in Table 2-5. To predict a selection gain for bones, meat and fat it is necessary to know variability and heritability of them. Following analizys (Table 2) showed no statistical differences for bones between L,Y,D and H. Signifacntly lighter bones was found in P. At the same time P had significantly less fat than all other breeds. D and H as terminal breeds also showed significantly higher more meat in carcass then Y and L. No difference in fat content between Y,L,D and H. Variation measured by coefficient of variation were similar in all analyzed groups. Similar values were presented by *Knap et al. (1994)*, *Taylor et al. (1982)* and *Appel et al. (1982)*.

Table 2. Yeald of bons and meat in carrcas at different breeds

Breed	Traits					
	Bone		Meat		Fat	
	$\bar{x}$	V%	$\bar{x}$	V%	$\bar{x}$	V%
Landrace	7,32	8,36	43,92	7,17	23,20	13,88
Yorkshire	7,54	9,38	44,54	7,34	22,10	13,72
Duroc	7,84	9,13	46,11	8,13	23,12	13,91
Pietrain	5,34	9,82	52,10	7,90	15,16	12,00
Hampshire	7,68	7,84	46,14	7,41	21,52	14,91

Daily gain were similar in all groups, except in Pietrain was significantly lower. Same tendencies were at a life gain as well. Variations were very similar in all breeds which open possibility for selection effect (Table 3).

Heritability estimated values (Table 4) were significantly different between traits. Highest were for meat yield (0,54) and gain (0,34) and lowest for bones yield (0,23). Genetic variation has been very similar. These facts may lead breeders to optimal improvement combining a selection and crossbreeding strategy on a long period run breeding program. Genetic parameters are very similar to one estimated by *Bergzma et al. (2010)*, *Rotschild (1990)*, *Brascamp (1985)* and *Park and Kim (1996)*.

Table 3. Life and daily gain at different breeds, g and variation, %.

Breed	Traits			
	Daily gain,g		Life gain,g	
	$\bar{x}$	V%	$\bar{x}$	V%
Landrace	823	10,3	711	11,6
Yorkshire	837	9,7	719	12,11
Duroc	843	10,9	723	11,4
Pietrain	672	12,4	534	14,9
Hampshire	803	12,0	690	14,0

Table 4. The heritability estimates for yeald of bones, meat content and life gain

Traits	h²	SEh²	VA
Meat	0,57	0,19	11,8
Bons	0,23	0,14	11,6
Gain	0,34	0,18	12,3

Genetic and phenotypic correlations (Table 5) are similar value and have the same tendency. Heavier bones means at the same time more meat in carcass. So the purpose of breeders is to select on bones capacity to improve total yield of carcass, especially meat yield. Similar estimates have been provided by *Vidović et al. (2011)*.

Table 5. Genetic (above) and phenotypic (under diagonal) corelations between certain traits

Traits	Bone	Meat	Fat
Bons	-	0,56	0,37
Meat	0,46	-	-0,76
Fat	0,23	-0,79	-

Conclusion

Daily and life gain has been very similar in L,Y,D, and H, also significantly lower in P. These are consequences of lower capacity in P and lighter bones weight at same breed.

The weights of bones were significantly less in Pietrain compare to other breeds. At the same time gain were also significantly lower and more cost in production for P.

The variations for analysed traits were similar.

Genetic and phenotypic correlation were expected size and showed a possible effect of indirect selection.

The heritability estimates are large enough to provide optimal selection effect and positive trends. Optimal breed structure and selection criteria for specialized breeds can provide genetic trend improvements of a trait we analyzed.

Selekcija na veličinu kosti i prinos mesa u svinja

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Rezime

Veleičina kosti je direktno korelirana osobina sa kapacitetom individue te prinosom mesa, prirastom ili pak profitom. Kako bi utvrdili ovakvu zakonitost ili analizirali mogućnost selekcije na ovako važna svojstva postavili smo višegodišnji ogled na 5 farmi svinja koje poseduju plodne rase: landras i jorkšir te terminalne rase: durok, hempšir i pietren. Kako su istraživanja trajala više godina, na više farmi i sezona, koristili smo metod najmanjih kvadrata maksimalne verovatnoće za korekciju sistematskih uticaja na ispitivane osobine. Kao fiksi faktor analizirane su i razlike između rasa. Za ocenu genetskih i fenotipskih parametara koristili smo polusestarske skupine po ocu. Disekcija polutki izvedena je po modelu EU, 1992. Ukupno je u istraživanja bilo uključeno 77 očeva, 2.136 majki, 6.151 potomaka u tovu i 955 disekciranih grla. Nastojali smo imati 2-3 direktna srodnika u delu disekcije čime se povećala tačnost ocene dobijenih rezultata i genetskih parametara.

Može se konstatovati da P ima sitnije kosti, da sporije raste u odnosu na ostale rase, te da su ustaovljene razlike signifikantne. Uprkos ustanovljenim razlikama varijabilnost ispitivanih svojstava između rasa bila je ujednačena. Ovo ukazuje na mogućnost daljeg efikasnog pritiska selekcije na poboljšanje ovih svojstava.

Heritabilnost i genetske korelacije su u granicama očekivanih vrednosti. Najniže ocene heritabilnosti ustanovljene su kod prinosa kosti, najviše kod mesa, a

negde između kod udela masnoće u polutkama. I ovde se pokazala veoma slična varijabilnost za ispitivana svojstva. Ova činjenica daje nadu za dalji prodor i promenu srednje vrednosti primenom optimalnih kriterijuma selekcije.

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NICKEL LEVELS OF LIVER FROM TEN DIFFERENT PIG GENETIC LINES PRODUCED IN VOJVODINA

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Abstract: The content of nickel was investigated in the liver of sixty-nine pigs from ten different genetic lines, produced in Vojvodina. Nickel was determined by the flame atomic absorption spectrometry after mineralization by dry ashing. The difference in the nickel content among different genetic lines of pigs was not significant ($P > 0.05$) in the analyzed liver tissues. Nickel levels ranged from 13.02 to 68.21 μg/100g with a general average of 26.73 μg/100g. Average nickel content, found in this study, is in agreement with the contents observed in pig liver in other countries.

Key words: nickel, liver, pigs

Introduction

Meat quality is the sum of all sensory, nutritive, hygienic-toxicological and technological factors of meat. The nutritive factors of meat quality comprise proteins and their composition, fats and their composition, vitamins, minerals, utilization, digestibility and biological value *(Hofmann, 1990; Honikel, 1999)*.
Red meat (beef, veal, pork and lamb) contains high biological value protein and important micronutrients including iron, zinc and vitamin B12, all of which are essential for good health throughout life *(Higgs, 2000; Williamson et al., 2005; Lombardi-Boccia et al., 2005; McAfee et al., 2010)*. Also, meat contains useful amounts of copper, magnesium, cobalt, phosphorus, chromium and nickel *(Higgs, 2000)*.

In most food products, the nickel content is less than 0.5 mg/kg fresh weight. Cacao products and nuts may, however, contain as much as 10 and 3 mg/kg, respectively *(IARC, 1990)*. According to *Solomons et al. (1982)*, dry beans, cacao products, baking soda, and some nuts contain high levels of nickel (>2.0 μg/g); wheat and wheat products, shellfish, processed meats and many vegetables

contain intermediate levels (0.2-2.0 μg/g); and whole and dried milk, fresh fruits, meat and eggs contain low levels of nickel (<0.2 μg/g).
Nickel is widely distributed in animal tissues in concentrations generally between 0.01 and 0.2 mg/kg (wet weight) when dietary nickel is not excessive (<25 mg/kg). Nickel does not accumulate with age in any organ, but, as with other mineral elements, overcoming homeostatic mechanisms by the addition of solube nickel salts to drinking water or diet elevates tissue and blood nickel concentrations *(NRCNA, 2005)*.

Nickel is present in a number of enzymes in plants and microorganisms. In humans, nickel influences iron absorption and metabolism, and may be an essential component of the haemopoietic process. COMA (Committee on Medical Aspects of Food and Nutrition Policy) and FDA (US Food and Drag Administration) were unable to set recommended amounts for nickel intake. Based on extrapolation from animal data, the hypothetical human requirement for nickel would be 16 to 25 μg/1000 kcal or about 75 μg of elemental nickel per day *(Solomons et al., 1982)*. Nickel deficiency has not been observed in humans *(EVM, 2003)*.

On the other hand, acute nickel exposure is associated with a variety of clinical symptoms and signs which include gastrointestinal disturbances, visual disturbance, headache, giddiness, wheezing and cough. Approximately 7-10% of the population (predominately women) are affected by nickel allergic dermatitis *(EVM, 2003)*.

The lowest reported oral dose associated with acute effects in humans was 0.05 mg/kg bw (1.2 mg in a 60 kg adult) *(EVM, 2003)*. Total diet studies indicate a total average oral intake of 200–300 μg/day *(WHO, 1991)*. Early estimates of daily nickel consumption in the USA ranged from 300 to 600 μg *(Schroeder et al., 1962)*. Recovery studies indicate an absorption rate of less than 15% from the gastrointestinal tract *(Sunderman et al., 1989)*. Dietary intake of nickel in food is not expected to result in harmful effects *(EVM, 2003)*.

The Autonomous Province of Vojvodina is a region where the number of animals of the porcine species and the production of pork meat are of high economic importance. Most studies have focused on the proximate compositions, vitamins and other essential nutrients. In the present investigation we determined the content of nickel in liver obtained from two pure and eight crossbred pigs used nowadays in Vojvodina for pork production.

Materials and Methods

Animals, sampling and preparing. The pigs used in the present study were produced in a pig (cross)breeding programme provided by nucleus and multiplication farms in Vojvodina (GGP-GP traditional pyramid structure of genetic programme) *(Visscher et al., 2000)*. In this breeding programme five pig

purebreds were used. The Large White (LW) and Landrace (L) were used as female lines and Duroc (D), Hampshire (H) and Pietrain (P), were used as male lines. An investigation was performed on sixty-nine pigs (castrates males and females) from ten different genetic lines (two purebred and eight crossbred pigs): [LW, *n* = 8; L, *n* = 7; LWxL, *n* = 7; LxLW, *n* = 6; Dx(LWxL), *n* = 7; Dx(LxLW), *n* = 6; (DxP)x(LWxL), *n* = 8; (DxP)x(LxLW), *n* = 7; (HxP)x(LWxL), *n* = 6; (HxP)x(LxLW), *n* = 7].

The pigs were randomly selected at an individual live weight between 95 and 110 kg and about six months old. One pig from each genetic line was taken at every six mounts from the same farm.

All the pigs were slaughtered in the two biggest Vojvodian slaughterhouses according to routine procedure. Carcasses and offal (liver) were conventionally chilled for 24 h in a chiller at 2-4°C. The samples for chemical analysis taken after the homogenization of the whole liver, were vacuum packaged in polyethylene bags and stored at –40°C until analysis.

Analytical methods and quality control. The nickel (Ni) content of the liver was determined after dry ashing mineralization according to the following procedure *(Gorsuch, 1970; Tomović et al., 2011)*: a twenty-gram sample was weighed into a porcelain crucible and dried in a laboratory oven at 105°C for 3 h. After drying the sample was charred on a hot plate and then incinerated in a muffle furnace at 450°C overnight (16 h). When a suitable ash was obtained it was moistened with little water, treated with 10 ml of hydrochloric acid/deionized water (1:1, v/v) and evaporated to dryness. Finally, the ash was redissolved with 10 ml of hydrochloric acid/deionized water (1:9, v/v), transferred into a 25 ml volumetric flask and diluted to volume with deionized water.

Nickel was measured in the ash solution by flame atomic absorption spectroscopy according to the manufacturer's instructions *(Varian, 1989)*.

A strict analytical quality control programme was employed during the study. The quality control of the analytical measurements for Ni was performed using the standard reference material (SRM): SMRD 2000 (Matrix meat reference material, National Food Administration, Uppsala, Sweden). For the determination of the Ni content the SRM samples were spiked with three different concentrations of this element. The results of the analytical quality control programme are presented in Table 1. In every series of samples, 2 blanks and 2 samples of standard reference material were included. All analyses were performed in duplicate.

Table 1. The results of the analytical quality control programme (n = 8) used in the determination of the nickel in pig liver

Ni	Recovery (%)	Limit of detection (µg/100g)
	103.2	12.5

Statistical analysis. All data are presented as mean, standard deviation (SD) and range. The analysis of variance (one-way ANOVA) was used to test the hypothesis about differences between more mean values. The software package STATISTICA 8.0 was used *(StatSoft, Inc., 2008)* for analysis.

Results and Discussion

The average content, standard deviation and range for the Ni in the investigated samples of the liver tissue of ten different genetic lines of pigs are presented in Table 2.

The order of the genetic lines of pigs according to nickel content in the liver samples (Table 2) in μg/100 g was: LWxL < Dx(LWxL) < (HxP)x(LWxL) < (DxP)x(LxLW) < LxLW < Dx(LxLW) < (HxP)x(LxLW) < (DxP)x(LWxL) < L LW. The content of nickel found in the present study did not differ significantly ($F = 1.244$; $P = 0.292$) among the liver tissue belonging to different genetic lines of pigs (Table 2). On the other hand, animals belonging to the same genetic line, from the same farm, raised under the same conditions, given the same feed, and slaughtered at the same age had Ni content in the liver that could differ up to four times (Tables 2). The lowest, average and highest nickel content in the liver was 13.02 [genetic line of pigs: Dx(LxLW)], 26.73 and 68.21 [genetic line of pigs: (HxP)x(LxLW)] μg/100 g, respectively. According to *Greenfield* and *Southgate (2003)*, biological material exhibits natural variations in the amounts of nutrients contained and the limits of natural nutrient variation are not defined.

The nickel levels obtained in pig liver in this study parallel those reported in the literature: Finland (< 20 μg/100g, *Nuurtamo et al., 1980*), Sweden (11 μg/100g, *Jorhem et al., 1989*), Denmark (17 μg/100g, ranged from 14 to 139 μg/100g, *Larsen et al., 2002*) and Spain (9 μg/100g, ranged from not detected to 31 μg/100g, *Lopez-Alonso et al., 2007*). Not much data are available for nickel content of pig liver in many countries.

Table 2. Nickel content (μg/100g) of the liver from the pigs in Vojvodina

Genetic line of pigs	Mean ± SD	Range
LW (*n* = 8)	35.71 ± 14.23	20.80–58.54
L (*n* = 7)	35.43 ± 15.08	13.91–55.00
LWxL (*n* = 7)	18.12 ± 4.94	14.59–27.72
LxLW (*n* = 6)	25.96 ± 4.93	19.77–31.61
Dx(LWxL) (*n* = 7)	20.37 ± 5.88	13.41–27.05
Dx(LxLW) (*n* = 6)	26.18 ± 11.72	13.02–38.28
(DxP)x(LWxL) (*n* = 8)	28.92 ± 12.57	18.32–53.11
(DxP)x(LxLW) (*n* = 7)	23.81 ± 10.37	13.28–43.77
(HxP)x(LWxL) (*n* = 6)	23.68 ± 13.11	13.97–45.62
(HxP)x(LxLW) (*n* = 7)	28.44 ± 21.62	14.63–68.21
All animals (*n* = 69)	26.73 ± 12.83	13.02–68.21

Conclusion

The results of the present investigation show that the content of nickel determined in the liver of pigs was not influenced by the genetic lines. Compared with developed countries, the nickel content in the liver tissue of pigs from Vojvodina parallel those reported in the literature. In addition, the obtained nickel composition could be used to provide regular nutrient compositional data of the pork meat in Serbia.

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Sadržaj nikla u jetri deset različitih genotipova svinja odgajanih u Vojvodini

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Rezime

U ovom radu određen je sadržaj nikla u jetri (n = 69) deset različitih genotipova svinja odgajanih u Vojvodini. Sadržaj nikla je određen plamenom atomskom apsorpcionom spektrofotometrijom nakon "suvog spaljivanja" uzoraka. Sadržaj nikla u tkivu jetre nije se značajno razlikovao ($P > 0.05$) između različitih genotipova svinja. Određeni sadržaj nikla bio je u granicama od 13,02 do 68,21 µg/100g, sa prosečnim sadržajem od 26,73 µg/100g. Prosečni sadržaj nikla, određen u ovom ispitivanju, odgovara sadržaju nikla utvrđenom u jetri svinja odgajanih u drugim zemljama.

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PALATABILITY AND EFFICACY OF RB SOFT BAG FORMULATED BAITS IN CONTROLLING HOUSE MOUSE AND NORWAY RAT IN ANIMAL FOOD BLENDER FACILITIES AND PIG FARM

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Original scientific paper

Abstract: The Norway rat and house mouse the most important rodent pest at farms eat and polullut the huge food quantity and carry a range of severe infectious deseases to humans and pets. Rodent control is undertaken only after its presence has been registered. Preparations for their control need to meet palatability requirements. Experiments were conducted in animal food blender facilities and pig farm (maternity and rooms with young and adult pigs) in Omoljica following the standard EPPO method. Palatability and efficacy of RB formulated baits (paste in special 10 g paper bags) containing 0.005% bromadiolone and 0.005% brodifacoum were tested against the house mouse and Norway rat . Baits in pelllete formulation were used as the standard. All the baits were placed in special boxes. Rodent abundance was evaluated based on the highest and the lowest daily baits consumption divided by the daily required of food amount, and a census method before and after treatment. Rodent presence was monitored over the next 20 days. The efficacy of the tested RB soft bag and standard formulations was calculated according to Abbott's formula. The results showed that palatability and efficacy of RB soft bag formulations (81-100%) was substantially high especially of brodifacoum-based baits. .

Key words: house mouse, Norway rat, RB soft bag formulation, palatability, efficacy, pig farm

Introduction

House mouse (*Mus musculus*) is one of the commonest rodent species. It reproduces rapidly. Namely, young females can breed at 6 time per year and produce numerous litters with up to 2000 baby mice a year *(Wilson and Reeder, 1992)*. Mice are omnivorous and may cause significant damage in both household

and farms. Rats are also among the most distributed rodent species. In Serbia the grey rat (*Rattus norvegicus*) is the commonest wich couses the serious damage, not only through consumed foood quantitty, an adult rat may eat 10,9 kg annually, but also through pollution and contamination up to nine time fold more of foood *(Kataranovski at al., 1988; Dunđerski at al., 1999; Djukić et al., 2005)*. Rodents have important epidemic significance as natural source and main vector of pathogens of animal and human origin or indirectly by numerous ectoparasites *(Gratz, 1988; Morita, 1995; Stajković at al., 1995; Leirs, at al., 2004; Olumide, 2008; Kyriakis and Alexopulos, 2011)*. In our country it is recorded several cases of trichinelosis and hemorragic fever wich were quite often ended with death. Two interesting cases were occured on pig farms in Vojvodina in 1995 year. At the one farm died 30, while at the other died 60 tons of rats wich were colllected, mixed with the oil and burned *(Radosavljević, 2003)*.

It is impossible to destroy rodents and for this reason their number should regularly be kept under control. Prior to that their number needs to be evaluated and the species present should be determined using the method of transect C-30 (WHO) *(Djukić et al., 2005)*, i.e. census *(EPPO, 1999)*. According to the HACCP system principles *(Kyriakis and Alexopulos, 2011)* strategy should be used to prevent damages due to rodent activity that includes several measures.

Preventive: agricultural practices, technological, manipulative, sanitary, hygienic, constructional, technical. Direct: mechanical, physical, biological, genetic and chemical methods. Mechanical: Application of different traps (baits, glues) (*Leirs et al., 2004; Singleton et al., 2004; Olumide, 2008)*. Biological: Use of parasites, prey birds or pathogenous microorganisms. However, caution is needed because accidental poisoning of man and useful organisms is not excluded *(Рилников and Тошигин, 1990.)*. Chemical Control: The rodenticides mostly used contribute to the most efficient and rapid reduction of overpopulated rodent pests. This is not always justifiable from the biological standpoint, especially acute rodenticides which provoke rapid death and hostility towards the bait offered, changes in the behaviour of the rodent, danger to non-target animals, man and the evironment *(Claperton, 2006; Fisher, 2006)*. There is no ideal rodenticide toxic to rodents only and non-toxic to animals and man. For this reason there is a big choice of rodenticides differing with regard to chemical composition, mode of action and toxicity *(Almaši, 2004; Vukša, 2008)*. Anticoagulant rodenticides of the first and second generation with the mode of action based on preventing the factors of blood coagulation are mostly used, and lethality is registered after a couple of days only. In the study conducted by Quy et al., a decrease in the palatability and efficacy of bromadiolone, difenacoum and brodifacoum baits was registered *(Quy et al., 1992)*. Therefore, it is necessary to develop acceptable preparations meeting palatability requirements that would stimulate rodents to choose baits rather than other food available in abundance in storehouses, prevent any hostility, protect non-target organisms and the environment .

Materials and Methods

Harmful species. Domestic mouse (*Mus musculus*) and Gray – Norway rat (*Rattus norvegicus*)

Localities. Experiment with *R.norvegicus* was conducted at rooms with adult pigs, dimensions that were 40 x 100 m, in pig farm " OMO RATAR", Omoljica and encompasses the surrounding area with active rodent holes.

Refferent examinations were conducted in maternity dimensions that was 50 x 30 m, lavatory room dimensions that was 5 x 6 m and rooms with piglets dimension that were 5x 7 m.

Experiment with Mus musculus in animal food blending facility pig farm " OMO RATAR", Omoljica dimension that was 490 x 30 m with blending machines and electrical installations.

Refferent examinations for *Mus musculus* were conducted in storage of animal food blending facility dimension that was 20 x 30 m with food stored in sacks lifted up on pallets.

Preparation studied. Palatability and efficacy of the following preparations was studied:

- Brodifacoum-based active substance BRODY FRESH BAIT (0,005 % brodifacoum) manufactured by KOLLANT, Italy, represented in Serbia by MAGAN YU, Subotica. Referent preparation applied was RATTACK-pellets (0,005 % brodifacoum) produced by DUOCHEM, Belgrade, Serbia.

- Bromadiolone-based active substance: RATIBROM 2 FRESH BAIT manufactured by KOLLANT, Italy, represented in Serbia by MAGAN YU, Subotica; GARDENTOP manufactured by ZAPI, Italy, represented in Serbia by AGROMARKET, Kragujevac; GLODACID PLUS manufactured by UNICHEM, Slovenia, represented in Serbia by PALPROPROM, Batajnica, Serbia and BRODILON MEKI MAMAC (BRODILON SOFT BAIT) manufactured by VETERINA, Kalinovica, Croatia. All preparations had 0,005% bromadiolone as active substance. Referent preparation was pellet formulation (0,005 % bromadiolone) HeMus AB produced by HEMOVET, Novi Sad,Serbia.

All the tested preparations were formulated as RB (ready to use) soft bait paste kept in special 10 g paper bags.

Trial methods. The trial complied with the PP 1/114(2) method *(OEPP/EPPO, 1999)*. Rodent abundance was assessed using the C-30 method (World Health Organization) or the transect method *(EPPO, 1990; EPPO, 1992)* at the beginning and 10 days after the beginning of the trial. Rodent abundance was evaluated based on the highest and lowest daily food consumption of baits over a 10 day trial period divided by the daily required amount of feeding. Placebo bait was laid at 10 spots in each facility over a period of 5 days.

At the allfarm facilities and the animal feed blending facility, on places where rodent activity had been observed previously and along rodent paths

(underneath pellets), tested and referent baits were placed in special boxes for bait exposition at 1-3 m intervals, one bag (10 g) for mice (*Mus musculus*) and three to five (30-50 g) for rats *(Rattus norvegicus*). Each box was marked with labels (with serial number), amount of preparation in (g) and the date of experiment . The duplicate of each label was sticked on the wall above the box, at the visible place, with warning elements according the HACCP standard regulations *(Bokelman, 1996)*.

Tested and standard baits were placed directly at the active holes, in amount of 3-5 bags (30-50 g), for Norway - grey rat found around the farm.

Effect mark. The control of bait consumption was daily conducted within 10 consecutive days. Rodent presence was monitored over the next 30 days.

Statistical methods. The palatability and efficacy of the tested RB soft bait and standard formulations was calculated according to *Abbott's (1925.)* and *Handerson-Tilton´s (1955.)* formula.

Rodenticides application. In the experiment in rooms with adult pigs 14 boxes with tested baits in amount of 3-5 bags (30-50 g) were placed at the distance of 2-3 m against *Rattus norvegicus*. The boxes were placed along the wals and the room doors to prevent pigs contact with preparation. Baits were also placed in the active holes along the outdoor wals of pigsty (pen).

In the refferent experiments against *Rattus norvegicus* at the maternity were placed 15 boxes with 3-5 bags (30-50 g) of RATTACK pellets at the distance of 3-5 m. At the maternity lavatory and rooms with piglets were placed 14 boxes at the distance of 2-3 m with 3-5 bags (30-50 g) of refferent bait HeMus AB.

In the animal food blending facility were placed 15 boxes with one bag (10 g) of tested preparations at the distance of 2-3 m.

In the refferent experiment against *Mus musculus* in the storage of the animal food blending facility were placed 10 boxes with 1 bag (10 g) HeMus AB.

Results and Discussion

Palatability and efficacy of the preparations studied. Table 1. shows the overall bait consumption, evaluated abundance of house mouse (*Mus musculus*) and Norway-grey rat (*Rattus norvegicus*) (at the start and at the end of trial) and efficacy in rodent control of standard and tested RB soft bait preparations on the basis of bromadiolone.

Table 1. Amount of bait on the basis of bromadiolone taken (g), rodent numbers and rodenticide efficacy in food blender facility and pig farm

Preparation (active substance bromadiolone)	Species	Bait taken (g)	Estimated number		Efficacy %
			Begining	End	
HeMUS AB (standard bait -pellets)	R. *norvegius*	116,0	8	1	97,50
	M. musculus	56,5	8	0	100,00
BRODILON MEKI MAMAC (RB SOFT BAIT)	*R. norvegicuss*	422,0	11	2	81,81
	M. musculus	356,0	37	4	89,20
RATIBROM 2 FRESH BAIT (RB SOFT BAIT)	*R. norvegicus*	491,0	10	2	80,00
	M. musculus	306,0	12	1	91,66
GARDENTOP (RB SOFT BAIT)	*R. norvegicus*	197,0	6	1	90,00
	M .musculus	250,0	25	1	96,00
GLODACID PLUS (RB SOFT BAIT)	*R. norvegicus*	1950,0	49	4	91,83
	M .musculus	244,5	25	1	96,00

The results showed high palatability of all the tested RB soft bait formulated preparations for house mouse (*Mus musculus*) and Norway-grey rat (*Rattus norvegicus*) compared with the referent pellet formulated preparation.

Our results showed high values (80,.00 do 97,50%) of all the tested RB soft bait preparations for the Norway-grey rat (*Rattus norvegicus*) control and very high efficacy (91,60 do 100 %) for the house mouse (*Mus musculus*) control.

The overall bait consumption, evaluated abundance of house mouse (*Mus musculus*) and Norway-grey rat (*Rattus norvegicus*) (at the start and at the end of trial) and efficacy in rodent control of standard and tested RB soft bait preparation on the basis of brodifacoum are shown in Table 2.

Table 2. Amount of bait taken (g), rodent numbers and rodenticide efficacy in food blender facility and pig farm

Product (active substance brodifacoum)	Species	Bait taken (g)	Estimated number		Efficacy %
			Begining	End	
BRODY FRESH BAIT (RB SOFT BAIT)	*R.norvegicus*	468,5	13	0	100
	M. musculus	70,0	14	0	100
Rattack pelets (standard baits)	*R.norvegicus*	372,5	8	0	100
	M. musculus	37,5	9	0	100

The results showed higher overall bait consumption of the tested Brody Fresh Bait preparation compared with the referent Rattack pellet preparation.

The Brody Fresh Bait preparation showed exceptional efficacy in both house mouse (*Mus musculus*) and Norway-grey rat (*Rattus norvegicus*) control.

Our trial results showed exceptional palatability of all the RB soft bait formulated prepations based on bromadiolone and brodifacoum as active substances.

The efficacy of tested bromadiolone-based preparations was extremly high (outstanding) (81,81 to 100 %), for the brodifacoum-based preparation was 100 % against *Rattus norvegicus* to 98.50 % against *Mus musculus* .

Based on some literature data (*Parshad et al, 1987.*), products based on bromadiolone and brodifacoum were found to demonstrate 80.8-97% efficacy against house mouse on poultry farms.

According to *Rowe et al. (1978)* brodifacoum efficacy against house mice on farms ranged from 92.7% to 100% (mean 98.8%). Bromadiolone efficacy against the same farm pest ranged from 92.7% to 100% (mean 92.4%) in another trial *(Rowe et al., 1981)*.

Our results in efficacy testing of products on the basis of bromadiolone and brodifacoum against house mouse (*Mus musculus)* and Norway-grey rat *(Rattus norvegicus)* are in accordance with those reported by other authors *(Rowe et al., 1981; Parshad et al., 1987; Brooks and Rowe 1987; Quy, 1992; Milić, 1999; Vukša et al., 2002; Leirs at al., 2004; Vukša 2008)*.

Conclusion

The Norway- grey rat (*Rattus norvegicus* Berk.) and house mouse (*Mus musculus* L.) are the most important pest rodents in food blender facilities and farms. It is very important to follow their abundance during the year and maintain it at the acceptable level, avoid losses and damage of storage facilities and prevent disease transmission.

To suppress rodents and maintain their number at the sustainable level is a delicate task and chemical control mesures are usually required.

The choice of baits should be given special attention considering rodents to be very intelligent and prudent animals but also the need to prevent environmental pollution. Baits which do not provoke fear and hostility (on the basis of anticoagulation active substances) and avoid environmental contamination should be applied .

Our investigation shows that the new RB soft bait formulation of rodenticide has exceptional palatability for both rodent species (*Rattus norvegicus* and *Mus musculus*) of major importance in storage facilities and outstanding efficacy of their control. It is also very convenient being packed in special paper bags which do not allow spilling and food and environmental contamination.

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Prihvatljivost i efikasnost RB mekog mamka formulacije u suzbijanju kućnog miša i sivog pacova u mešaoni stočne hrane i na farmi svinja

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Rezime

Suzbijanje glodara vrši se uglavnom po uočavanju brojnog prisustva. Preparati treba da imaju što bolju prihvatljivost.

Eksperimente smo obavili u mešaoni stočne hrane i na farmi svinja (obori sa odraslim svinjama, porodilište i prostorije sa malim prasićima) u Omoljici po standardnoj EPPO metodi. Prihvatljivost i efikasnost RB formulisanih mekih mamaka (u obliku paste u kesicama mase 10-20 g) sa 0,005% bromadiolona i 0,005% brodifakuma kao aktivne supstance su testirani za suzbijanje kućnog miša i sivog pacova. Kao standard koristili smo mamke u obliku peleta. Mamci su postavljani u specijalne kutije. Brojnost glodara izračunata je na osnovu najveće i najmanje količine pojedenog mamka podeljeno sa dnevnom količinom potrebne hrane, a primenjen je metod cenzusa pre i posle tretmana. Prisustvo glodara praćeno je i 30 dana posle tretmana. Prihvatljivost i efikasnost testirane RB (meki mamak) i standardne formulacije izračunata je prema Abott-ovoj i Handers-Tilton-ovoj formuli.. Rezultati su pokazali da je postignuta veoma visoka prihvatljivost testirane RB formulacije naročito za mamke na bazi brodifakuma kao aktivne supstance. Efikasnost svih testiranih preparata na bazi bromadiolona bila je izuzetno visoka, 81,81 do 100 %, a za preparat na bazi brodifakuma iznosila je 100 % i za *Rattus norvegicus* i za *Mus musculus*.

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APPLICATION OF NIR TECHNOLOGY IN THE ANIMAL FOOD INDUSTRY

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Abstract: The importance of NIR technology in the animal food industry is presented in this study. As the example of the calibration procedure of NIR devices a calibration model for 14 samples of soybean cake was designed. Samples were previously analyzed in the standard laboratory testing of the moisture content, content of crude proteins, crude fats and crude fibre. In this calibration procedure high determination coefficients - R^2 were established for these parameters of the nutritional value of food (0.9783 for moisture, 0.9904 for crude proteins, 0.9872 for crude fats and 0.9351 for crude fibre). The comparison of values obtained by using standard laboratory methods with values obtained by NIR technology/method indicates that by using NIR devices it is possible to obtain highly reliable results, and therefore it can be used successfully in facilities for production of animal food in the control of the quality and projection of mixtures.

Key words: NIR, animal food

Introduction

Adequate nutrition of animals includes knowledge of the characteristics of used feeds *(Jovanovic et al., 2009)*. Also, intensive rearing of farm animals and stringent requirements in regard to the quality and quantity of animal products, require constant monitoring of the chemical composition of food for animals. On the other hand, strong competition in the field of production of animal food, imposes higher efficiency of the production process. Results obtained in the standard chemical analysis methods of major quality parameters of raw materials and finished mixtures are often received too late to stop the production process on time or correct it *(Vries et al., 2010)*. Therefore, it is necessary to use fast analytical methods which enable fast response in cases when certain deviations are observed from the projected composition or quality of the product. In this way it is possible to achieve significant savings in the production process and ensure product of stable quality. NIR method of analysis is exceptionally fast (usual duration of

analysis is 10 seconds), non-destructive and does not require preparation of the sample for analysis.

Main principle of NIR method of analysis. NIR(S) method (Near Infrared Reflectance Spectroscopy) is based on screening of sample by using near infrared reflectance/light, resulting in the spectrum of this individual sample. Spectrum can represent dependence of the reflected or transmitted radiation from the wave length. Results of the indirect NIR method, i.e. spectrum data, subsequently have to be transformed into required results – concentration of the relevant constituent or functional property of the tested material, which is obtained by developing/designing of the calibration model. Calibration of NIR devices is done by using samples of known composition and properties determined by standard (reference) analysis methods. Calibration procedure includes application of certain mathematical and statistical techniques (chemometrics) for the purpose of obtaining of empirical equation which connects spectrum data with data obtained by chemical analysis (*Stuth et al., 2003*). The costs associated with chemical analysis of large number of samples (minimum 50, whereas approx. 150 chemical analyses of different samples is required for open calibration sample population) are typical for single chemometric method. Calibration of NIR devices requires certain amount of time, but it is facilitated by the possibility of sophisticated software packages which provide for the user application of chemometric calibration techniques (MLR Multiple Linear Regression, PLS – Method of Partial Least Squares Regression, PCR – Principal Component Regression, ANN – Artificial Neural Network, etc). However, there are multiple benefits/advantages provided by the NIR method which are beyond the time and money invested in its implementation.

Development of calibration model represent key step in successful implementation of NIR method and it comprises following phases:

- Selection of calibration set of samples; robustness and accuracy of the calibration model greatly depend on the variability of the calibration population in sense of presence of samples of different varieties, samples of various maturity stages, samples originating from different cultivating regions and different production years (*Tsuchikawa, 2007*);
- Collection of spectrum and reference data;
- Execution of the regression (calibration) model and,

Validation of the model; objective of the calibration model validation is to assess its predicting abilities in a routine application (*Petersen, 2007; Boysworth and Booksh, 2008*). We distinguish two types of validation procedure of NIRS calibration model; cross-validation and external validation, i.e. validation using an independent set of samples.

Both procedures result in prediction error, i.e. error which can be expected in a routine application of model used to determine the quality, i.e. efficiency of the developed model (*Esbensen, 2006*), and which is based on the concept of

differences between NIR results and results of reference laboratory analyses (*Isaksson and Segtnan, 2007; Shenk et al., 2008*).

Materials and Methods

Samples of soybean cake were analyzed using standard chemical methods (reference methods) and NIR method, in order to compare results of these two methods. Analyses were done in FSH Komponenta from Ćuprija. The analysis of the moisture content, content of crude fats and proteins was done on 14 samples, and analysis of crude fibre content on 9 samples of mentioned feed. All analyses were done according to the Rulebook on methods of physical, chemical and microbiological analysis of animal food from year 1987. Analysis of the moisture content was done on the laboratory moisture - meter OHAUS® type MB45 for fast determination of moisture content. Chemical analysis of crude proteins was done according to method by Kjeldahl; for determination of the crude fats content the procedure of dry extraction according to Soxhlet method was applied, using VELP® Scientifica digester and fat extraction apparatus. In chemical analysis of crude fibre the Weender method was used. Chemical analysis was done in two repetitions and average value of obtained results was considered in the study. For NIR analysis of samples NIR device was used - PERTEN Diode Array 7200, with rotating dish for measuring of samples, of diameter of 75 mm. This type of NIR device does not require previous grinding of samples, which is also an advantage. The wave length range from 950 to 1650 nm was used for measuring. The following mathematical transformations were carried out, on the spectrum of the soybean cake sample, in order to remove all irrelevant information from the spectrum, and to be able to interpret the results easier: Savitzky-Golay – to obtain first spectrum excerpt and MSC – Multiplicative scatter correction, and for the purpose of development of calibration model, PLS1 – Partial Least Squares Regression. For transformation of the main spectrum of the soybean cake samples and execution/development of calibration model software package CAMO Unscrambler® 10.1 was used.

Results and Discussion

In assessment of the efficiency of the obtained calibration model the following statistical tests were used: RMSEP – Root Mean Square Error of Prediction and R^2 – Determination coefficient R^2 (Table 1). Root Mean Square Error of Prediction – RMSEP represent measure of the variability of differences between values obtained by NIR analysis (predicted values) and reference laboratory methods. When the value of RMSEP is closer to the zero, the calibration model is more reliable. Determination coefficient R^2, represents the square of the correlation coefficient – r, and describes the variation and range of the calibration

set. Value of R^2 = 1, means that 100% of variations are described with the calibration *(Pojić, 2010)*. Based on presented results it can be concluded that very good determination coefficients (R^2) in all cases are obtained. In comparison of concentration values of tested parameters obtained by standard laboratory method and those obtained by NIR method (Table 2), it is observed that results of the NIR method of analysis show no significant deviations from results obtained by standard laboratory methods, and errors which occurred did not exceed deviation regulated in the Rulebook on quality and other requirements for animal food („Official Journal of RS", no. 4/2010). The highest determination coefficient (R^2) was achieved in crude proteins, which was expected since N-H chemical bond present in proteins shows high level of absorption of infrared radiation *(Oatway et al, 2006)*. Very good determination coefficient (R^2) was also achieved in crude fats, although the concentration range of this constituent was considerably wide. Slightly lower determination coefficients (R^2) were obtained fro moisture and crude fibre. On the other hand, value of the Root Mean Square Error of Prediction - RMSEP was the lowest in crude fibre (lower than in crude proteins) which is explained by very narrow range of content of this parameter.

Table 1. Concentration of crude proteins and crude fats in the calibration set (%), range of their values and results of the statistical cross validation

	Average of values, %	SD	Range	R^2	RMSEP
Moisture	7.31	1.52	4.06 – 9.65	0.9783	0.2442
Crude proteins	38.12	0.53	37.29 - 39.15	0.9905	0.0517
Crude fats	7.95	1.21	6.49 - 11.31	0.9884	0.0972
Crude fibres	6.02	0.32	5.72 – 6.91	0.9351	0.04331

SD – Standard deviation, R^2 – Determination coefficient, RMSEP – Root Mean Square Error of Prediction.

Table 2. Comparative review of the results of standard laboratory and NIR methods of analysis

Sample	Moisture		Crude proteins		Crude fats		Crude fibres	
	St.l.m, %	NIR, %	St.l.m, %	NIR, %	St.l.m, %	NIR, %	St.l.m, %	NIR, %
1	4.060	4.446	37.780	38.669	8.880	8.761	5.720	5.742
2	7.540	7.752	38.140	38.202	7.000	7.131	6.080	6.001
3	7.000	6.849	37.720	37.674	8.880	8.985	5.900	5.927
4	8.080	8.373	37.810	37.816	7.180	7.082	5.880	5.882
5	8.160	8.047	37.400	37.391	7.290	7.406	5.920	5.952
6	9.130	8.921	37.330	37.349	6.490	6.533	5.680	5.705
7	7.130	7.143	37.800	37.798	8.090	8.061	6.020	6.002
8	8.160	8.266	37.770	37.791	7.950	7.858	6.100	5.952

St.l.m – Value obtained by standard laboratory method
NIR – Value obtained by NIR method of analysis

During the process of development of calibration model and its validation, certain number of samples had to be excluded in order to obtain the most reliable model possible. Therefore, the number of samples presented in Table 2 is lower than initial number of samples of soybean cake (9 samples for crude fibre and 14 samples for other parameters). This is also reason why, in case of moisture, crude proteins and crude fibre, a model was obtained which gives more reliable results, but with narrower range of contents of determined nutritional parameters. In case of crude fats, however, a calibration model was obtained which gives very reliable results for wide range of concentrations of this constituent. Based on obtained results it can be concluded that even with small number of samples which were tested by using standard laboratory methods of analysis, it is possible, using NIR method, to receive very reliable results, but adequate selection of samples for development of calibration model is of crucial importance. When the number of samples with various concentrations of relevant constituents is higher, a calibration model for wider range of concentrations can be developed, which would also give reliable results.

There are also other ways of implementation of NIR technology, such as use of calibrations offered/provided by manufacturers of NIR devices. However, this way is associated with considerable costs. Also, calibration curves provided by manufacturers of NIR devices, although developed on large number of samples, often need to be adjusted due to high variations in the quality of raw materials.

Conclusion

Introduction of NIR technology is a good strategy of development of facilities for production of animal food, if the complexity of their production process is taken into consideration. NIRS method, as fast analytical method, enables timely receiving of required results, improvement in the quality control and increase in the efficiency of the animal food production, wit final outcome in increase of the profitability and competitiveness.

Results obtained by applying NIR method in analysis of the soybean cake samples showed no significant differences compared to results obtained by standard laboratory methods, which proves that NIR method can be very reliable in determination of the composition of raw materials used in production of animal food, as well as of finished mixtures. Also, duration of NIR method of analysis was incomparably shorter than duration of standard laboratory methods.

Primena NIR tehnologije u industriji hrane za životinje

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Rezime

Sve oštrija konkurencija u domenu proizvodnje hrane za životinjezahteva da proces proizvodnje bude što brži i efikasniji. NIRS metoda, kao izuzetno brza analitička metoda, predstavlja dobru soluciju koja poseduje veliki potencijal za unapređenje nadzora i kontrole proizvodnog procesa u fabrikama hrane za životinje. Uzorci sojine pogače analizirani su standardnim hemijskim metodama (referentne metode) i NIR metodom, u cilju poređenja rezultata ove dve metode. Analiziran je sadržaj vlage, sirovih proteina, sirove masti i sirove celuloze. U postupku kalibracije NIR uređaja za ove parametre kvaliteta sojine pogače, ostvareni su veoma dobri koeficijenti determinacije (R^2), što znači da su dobijeni pouzdani kalibracioni modeli. Rezultati dobijeni primenom NIR metode za analizu uzoraka sojine pogače (u postupku validacije modela), nisu se bitnije razlikovali od rezultata standardnih laboratorijskih metoda, što dokazuje da da NIR metoda može biti vrlo pouzdana u određivanju sastava, kako sirovina koje se koriste u proizvodnji hrane za životinje, tako i gotovih smeša. Prednosti uvođenja NIR tehnologije u fabrike hrane za životinje su: brza kontrola sadržaja relevantnih sastojaka u sirovinama i gotovim proizvodima, poboljšanje kvaliteta proizvoda, snižavanje troškova, mogućnost ugradnje NIR uređaja u proizvodni proces (at line analiza) - kontinualna kontrola procesa proizvodnje hrane za životinje.

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PRODUCTION RESULTS OF VARIOUS CATEGORIES OF PHEASANTS REARED UNDER CONTROLLED CONDITIONS

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Review paper

Abstract: The overview of domestic and foreign investigations of the effects of various intensity of ant feeding technique in various categories of pheasants. With the aim to increase number of pheasants in our country and in the world, for decades pheasant chicks are produced in pheasant farms up to the age of 5-8 weeks, and body mass of 400-450 g, after which they are released into the hunting grounds. The capacity of pheasant farms in Serbia is about 900.500 hatched chicks, and in the past four decades several tens of millions of artificially reared pheasants were released. The quality of feeding the brood stock of pheasants, which produce eggs for hatching incubator has a direct influence on number, mass and fertility of eggs, and on the mass of newly hatched chicks. The feed conversion ratio in chicks depends on the first place on energy and protein level, and also on biological value of protein. In domestic investigations of pheasant chicks feeding with the concentrate mixture with higher protein value (30% to 28 days of age and 24% to 42 days of age) and with lower stocking density (450 individuals in the group), had significantly bigger Final body mass (457.07 g) and higher daily gain (4.22 g in the first 13:31 and g in the second period), and with better feed conversion ratio.

Key words: pheasants, rearing, feedin, results.

Introduction

Recent years, in Serbia is recorded reducing the number of pheasants *(Popovic et al., 2011b)*. The main reasons for this are: significantly reduced range, all the worse natural conditions related primarily to food, the maximum mechanization of agriculture and chemical factors, different groups of predators in

the wild and domestic animals and others *(Popović, 2006a; Popović et al., 2008, 2009a)*. In search of food, pheasant sometimes causes damage to the crop and vegetable crops *(Popović, 2006b, 2007)*. Attempts to complementary food for pheasants in the countryside during the spring breeding period increased their numbers were almost no results *(Hoodles et al., 2001)*. Therefore, the controlled pheasant breeds in artificial conditions, like poultry, to a certain stage of growth after an appropriate adjustment, was released in the hunting area *(Carroll et al., 1997)*. Pheasant rearing technology consists of several segments: the parent breeding flocks, production of eggs for incubation and rearing chickens under controlled conditions until the age of 6 weeks *(Popović et al., 2011a)*. To achieve maximum results it is necessary to provide a series of important factors, such as appropriate facilities, controlled conditions and precise food at various stages of production and categories depends of age *(Pekeč, 2003)*. Nutrition in rearing object in the intensive cultivation at the beginning of pheasant chickens performed exclusively concentrates *(Popović and Đorđević, 2009)*. Later, in order to imitate the natural food, meals supplemented grain and green food *(Kokoszynski et al., 2008)*. Nutrition of the breeding flock is different depending on whether it is a season of wearing or not. In all stages of production listed there are some losses in the form of reduced capacity, poor quality eggs and fertility, and mortality of pheasant chickens. Improvements in the technology and growing percentage of chickens hatching is ever increasing to 50% brought up to the number of incubate eggs increased to 70% and in exceptional cases and in 75% *(Mantovani et al., 1993)*. Current capacity of the pheasant farm in Serbia's 900,500 one-day old pheasants *(Popović and Stanković, 2009)*. For the past four decades in Serbia hunting was put tens of millions of artificially growth pheasants *(Popović et al., 2009b)*.

Nutrition of the parent flock of pheasants and production results

Nutrition of parent flock has significant impact on the number of eggs *(Carey et al., 1980)*, their mass *(Usturoi, 2008)* and fertility *(Nowaczewski and Kontecka, 2005)*, and from the eggs with larger mass is hatch the larger pheasant chickens *(Ipek and Dikmen, 2007)*. They are different needs out pheasant egg laying period and during the season of wear. Primary need for pheasants in captivity 40-55 g amounts of grains or a simple mixture. However, the need for pheasant chickens and female of pheasant layers are significantly higher. Namely, in nature pheasant female to lay 12-18 egg masses of 28-29 g per egg, while in the aviary and to lay 60 eggs for about 3 months *(Popović and Đorđević, 2009)*. According to the *AEC (1987)* and *INRA (1984)* recommendations, the protein needs are less than 15%. However, *Hanuš and Fisher (1983*) indicate a much

greater need, that the dissemination of a minimum 18% crude protein in the diet, and in the course of carrying 20-25%. In practice, energy needs are provided with 60-70% of grain in the ration. Especially important is the level of calcium in the diet. *Greeley (1962)* states that similarly to poultry, the deficit of calcium in the diet of pheasant laying hens capacity decreases and cope with the weaker-shelled eggs.

In previous years, practiced the posture of the parent cluster in families, with sex ratio 1: 8 in favor of females. Today, the group applies the hold, which proved to be practical. In this way of holding capacity by 5 to 10% lower, but the percentage of fertilization are in range from 80 to 95% *(Popović and Stanković, 2009)*.

Preparation the parent flock of pheasants for the season it start in January, so pheasant will receive the same type of mixture which will be used in periods of wear. Mixture is first distributed twice a week, then three times in the period when the flock is divided into families or groups on a daily basis. After the formation and transmission groups (families) in the summer stalls, food is given to the automatic feeders, at will. During wear, daily intake of concentrated pelleted feed for pheasant laying hens is 80 g. Pellet size is 4 × 6 mm. In addition to food, it is necessary to provide sufficient amounts of water and sand (finer stones) for picking a more efficient digestion.

Table 1. The percentage of hatched to the number of eggs to lay *(Popović and Stanković, 2009)*

Pheasant farm	Year	Number to lay eggs	Percentage to lay out of eggs laid	Total number of hatched pheasant chicken during the season	Percentage hatched pheasant chicken of total to lay eggs in a season	Average number hatched pheasant chicken per pheasant femail per year
Vinik	2002nd	27,720	91.14	14,109	50.90^{b}	21.12
	2003rd	20,135	94.69	14,027	69.66^{a}	27.67
	2004th	24,172	99.30	14,800	61.23^{ab}	26.76
Rit	2002nd	25,000	55.87	17,700	70.80^{ns}	18.04
	2003rd	34,000	77.16	23,410	68.85^{ns}	23.89
	2004th	40,000	76.00	28,210	70.53^{ns}	24.24
Significance examined the impact of factors						
Year		-	-	-	P> 0.05	-
Pheasant farm		-	-	-	P <0.05	-
Year ×Pheasant farm		-	-	-	P <0.05	-

According *Urošević (2005)*, pheasant hatching in local conditions (about 90 days, the period April-June) is 41-45 eggs. Success depends on the incubation of the biological quality of eggs, incubators modes and engagement of people. Biological quality is related to eggs fertility, the manner and length of storage like and proper selection of eggs for hatching. To the fertility affect structure of the parent clusters, the method of holding and gender. The fertility can adversely effect the cold and rainy periods, the presence of unknown persons and other harassment of the parent flocks *(Popović and Stanković, 2009)*. Testing the pheasant farm in Serbia, „Vinik“ and „Rit“ just confirmed existence a large difference in the percentage of hatched chicks, as a result of these factors (Table 1).

Nutrition pheasant chicken and production results

Body weight at the time of their settlement in the grounds is very important for survival during the period of adaptation *(Pekeč et al., 2006, 2008; Đorđević et al., 2010, 2011)*. There are a series of recommendations for pheasant chicken nutrition, considerably different in the amount of certain nutrients *(Popović and Đorđević, 2009)*. Great attention is paid to the quality and quantity of protein *(Ohlsson and Smith, 2001)*. *Đorđević et al. (2010)* examined the effect of different levels of protein in the diet (A_1 = 26% by the end of the 4th week of life and 20% from the 4th to the end of sixth week of life, A_2 = 30% crude protein until the end of the fourth week of life and 24% by the end of the fourth week until the end of sixth week of life) and a variety of pheasant density (B_1 and $B = 450_2$ = 550 birds / group) on the performance and mortality (table 2). At the end of the experiment (42 days) at low density (450 birds per group) chicken pheasant weight in the first group (373.85g) was significantly lower than in the other group (457.07 g).

Table 2. Production results of pheasants chicks *(Đorđević et al., 2010)*

Proteins, %	Density, bird/group	Body mass, g			Daily liveweight gain, g/day		Conversion, kg/kg	
		Age, days			Period, days		Period, days	
		0	15.	42.	0-15.	15-42.	0-15.	15-42.
A_1 I period=26 II period=20	B_1 = 450	20.69^{a}	64.81^{a}	373.85^{ab}	2.94^{a}	11.03^{ab}	2.68^{ab}	3.31^{c}
	B_2 = 550	20.90^{a}	59.23^{a}	336.53^{a}	2.55^{a}	9.53^{a}	3.53^{b}	3.10^{bc}
A_2 I period=30 II period=24	B_1 = 450	21.08^{a}	84.32^{c}	457.07^{c}	4.22^{c}	13.31^{c}	1.81^{a}	2.75^{ab}
	B_2 = 550	20.40^{a}	72.88^{b}	408.04^{bc}	3.50^{b}	11.97^{bc}	1.97^{a}	2.51^{a}
Values for P								
A		0.82^{ns}	0.00^{**}	0.00^{**}	0.00^{**}	0.01^{**}	0.01^{**}	0.00^{**}
B		0.37^{ns}	0.01^{**}	0.03^{*}	0.02^{*}	0.06^{ns}	0.11^{ns}	0.07^{ns}
A × B		0.14^{ns}	0.29^{ns}	0.80^{ns}	0.42^{ns}	0.78^{ns}	0.24^{ns}	0.86^{ns}

In the experiment *Rizvanov et al. (1984)*, with breeding peasant chicken the concentrate mixtures were used with 25, 28, 30 and 35% crude protein. In addition, mass pheasant chicken after 50th days of life amounted to 401.3, 434.3, 452.3 and 451.2 g. This means that there is a physiological limit to the level of protein in the diet after the animals do not react further increased weight gain. Higher than the recommended level of protein is irrational, and may be harmful because it leads to pathological changes in liver and kidney *(Pekeč et al., 2006)*.

After the fifth week of life, pheasant chckhens given grains and green mass in order to adapt to the diet in nature. Breeding pheasant chicken ending about 60 days old when released for hunting. The period of settlement pheasant in a hunting ground is a time of great drought and harvest wheat, when must come a significant feed deficit. According to research in Ireland, about 70% of young birds in the 12 weeks of age die or disappear due to lack of food, parasitic infections, and predators *(Popović et al., 2010)*. Because of the increased mortality must be some time after the release of young pheasant hunting to continue in their feeding *(Sage et al., 2002)*. According to *Đorđević et al. (2011)* in this critical period should be provided daily 3-4 kg of food grain per 100 young birds, the feeding should be sure to offer water.

Conclusion

Lack of natural reproduction of pheasants require their replication in strictly controlled conditions, with very intense diet. In recent experiments, local capacity is determinate load egg of female pheasant in aviaries of 41-45 eggs for three-month period. The percentage of hatched pheasant chicken in regard to incubated egg are in the range of 50-70%. When feeding diets containing 30% crude protein (to 28 days), or 24% crude protein (from 29 to 42 days) achieved a weight of 457.07 g. However, it still remains the problem of high mortality of young birds on the hunting ground settlement due to lack of food, parasitic infections and predators. Therefore, the proposed management measures required as a hunting ground feeding 3-4 kg of grains per 100 young birds, as well as provision of water.

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Proizvodni rezultati različitih kategorija fazana gajenih u kontrolisanim uslovima

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Rezime

U radu je dat pregled domaćih i stranih istraživanja efekata različitog intenziteta i tehnike ishrane na proizvodne rezultate različitih kategorija fazana.

U cilju povećanja broja fazana za odstrel u svetu i kod nas decenijama se gaje fazančići u fazanerijama do starosti od 5-8 nedelja i telesne mase od 400-450 g a zatim se puštaju u lovišta. Kapacitet fazanerija u Srbiji je oko 900.500 jednodnevnih fazančića a za protekle četiri decenije u lovišta Srbije pušteno je nekoliko desetina miliona veštački odgajenih fazana.

Kvalitet ishrane matičnog jata fazana koji proizvode jaja za inkubatore direktno utiče na broj, masu i fertilitet jaja, kao i na masu fazančića. Konverzija hrane kod fazančića zavisi od nivoa energije i proteina, kao i od biološke vrednosti proteina, pre svega od sadržaja metionina i lizina. U domaćim ispitivanjima ishrana fazančića smešom koncentrata sa većim nivoom proteina (30% do 15. dana života i 24% do 42. dana) i pri manjoj gustini naseljenosti (450 jedinki u grupi) rezultovala je signifikantno većom završnom telesnom masom (457,07 g) i većim dnevnim prirastima (4,22 g za prvi period odgajivanja i 13,31 g za drugi period), kao i boljom konverzijom hrane.

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IN VIVO PREDICTION OF LIVE WEIGHT AND CARCASS TRAITS USING BODY MEASUREMENTS IN INDIGENOUS GUINEA FOWL

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Abstract: The objectives of this study were to evaluate the relationship between live measurements and carcass traits, and develop linear regression models to predict live weight and set of carcass traits in an indigenous guinea fowl. Twenty eight adult indigenous birds of both sexes were used for the study. Live weight and body measurements were obtained before slaughter while carcass traits were taken on hot carcass. Result obtained from descriptive statistics showed that, mean performance were 1208±6.86 g, 22.17±0.13 cm, 8.94±0.07 cm, 2.96±0.03 cm, 34.23±0.19 cm, 850.15±7.18 g, 267.23±1.69 g, 72.39±0.64 g and 70.38% for body weight, body length, thigh length, keel length, chest circumference, carcass weight, breast weight, thigh weight and dressing percentage. All the traits except for keel length were positively ($P<0.001$) correlated to body weight. Chest circumference had the highest predictive power in live weight estimate (R^2 .558), while body weight stand out as the single most important variable in carcass weight and breast weight prediction (R^2 .820 and .902) This suggest that that carcass weight and breast weight prediction can best be obtained using body weight, providing direction in developing model for selection and improvement of guinea fowl for meat production.

Key words: Guinea fowl, relationship , body measurement, carcass trait

Introduction

Guinea fowl (*Numidia meleagris*) originate from Africa, where it exist in large number in the wild *(Gracey et al., 1999; Saina, 2005)*. The production of guinea fowl as an alternative poultry is gaining ground throughout the world, especially in developing nations with increasing demand for its meat because of the advantage of the grainy flavour *(Mareko et al., 2006)*.

As a result of the increasing interest in farming guinea fowl and the gradual domestication of the bird, it is required to develop breeding strategies that will

bring about improvement in its performance and in supply of meat and egg. There are little or no available literatures on selection direction towards increasing live weight or carcass of guinea fowl for now . *In vivo* prediction of carcass component based on single trait are usually discouraged as not reliable. *Raji et al. (2009)* and *Wawro (1990)* proposed that more accurate results can be obtained when several parameters are used as independent variables in predicting and improving carcass performance in birds, this was substantiated when multiple traits where use in a regression analysis .

Carcass meatiness in poultry depends first of all on the components of breast and leg muscles *(Wilkiewicz-Wawro et al., 2003)*. Selection should be aimed at developing these areas. Body measurements can be useful in breeding work particularly in weight and carcass improvement *(Wawro and Wawro, 1989; Wawro 1990; Wawro and Jankowski 1990)*.

Most models were developed by multiple linear regression procedure where collinearity among the independent variables was not evaluated . However collinearity problem among independent variables should be expected as these are both genetically and phenotypically correlated *(Simm and Dingwall, 1989)* and it is known that model based on multicollinearity variable can limit inference and the accuracy of prediction *(Chatterjee et al., 2000)*. In fact the use of collinear variables as independent variables does not improve the model precision, and create instability in the regression coefficients estimation *(Shahin and Hassan, 2000)*.

The objective of this work was to evaluate the relationship between live measurements and carcass traits and to determine the usefulness of body measurements in predicting live weight and some carcass traits in guinea fowl.

Materials and Methods

Experimental animals and their management. Twenty eight indigenous guinea fowl made of thirteen males and fifteen females were bought from Kano main market and transported to Lafia. The birds were kept for two weeks at a rearing pen in the Teaching and Research Farm of College of Agriculture, Doma Road , Lafia , and were placed on optimum feeding for the period of their stay with concentrate diet containing 18% CP and a metabolizable energy of 2700 kcal/kg and are supplied with freshly clean water *ad libitum*. After two weeks, the birds were used for the analysis, body measurement were taken and carcass traits were measure after being starved for 12 hours from feed and slaughtered.

Parameter measured. Live body measurement include, body weight(BWT), body length (BL), wing length (WL), thigh length (TL), keel length (KL) and chest circumference(CC). While carcass measurement traits include carcass weight (CCW), breast weight (BRW), thigh weight (THW) and dressing

percentage .Kitchen scale and graduated measurement tape was used to obtain the data. To ensure accuracy , each measurement was taken twice , same person throughout took all measurements and weighing , thus eliminating error due to personal difference. The data from males and females are combined since there was no significant difference between the sexes in the above mentioned traits.

Statistical analysis. Data collected were analysed for preliminary descriptive statistics(mean±se, minimum , maximum and coefficient of variation). Pearson's correlation subroutine was used to determine the coefficient of simple correlation between live weight , body measurements and the target carcass components (carcass weight , breast weight and thigh weight). Sex effect was found not to be significant. Step wise multiple regression was performed to estimate live weight and carcass weight using body measurements traits to produce the best regression model for each dependent variable based on the regression coefficient(R^2). Step wise regression is a standard procedure for variable selection, which is based on the procedure of sequentially introducing the predictor into the model one at a time. It starts as the forward selection but at each stage the probability of deleting a predictor as backward elimination is considered. The number of predictors retained in the final model is determined by the level of significance accounted for inclusion and exclusion of predictors for the model (Chatterjee et al., 2000). Due to the influence of collinearity on the reliability of coefficient of determination (R^2) as outline by variance inflation factor , VIF was determine for each stepwise to ascertain the usability of the R^2 obtained (Rook et al 1998). The following was used as

$$VIF = 1 / 1 - R^2$$

Where

R^2 =coefficient of determination

Statistical package SPSS 14.0(2004) was used for the analysis

Results and Discussion

Means and their corresponding standard errors , minimum, maximum and coefficient of variation for all live body measurements and carcass traits are presented in Table 1. Wing length, chest circumference had the lowest variability, similarly the other traits had variability below ten percent, this might be as a result of breed identity and specificity indicating homogeneity of the population. The mean body weight obtained was 1208 g, comparable to what *Galor (1985)* and *Ayorinde (1991)* obtained for exotic guinea fowl reared in Nigeria. Though the

values recorded here were higher than what *Ayeni (1983)* and *Dahouda et al. (2009)* obtained from same strain of indigenous guinea fowl (1110 g) and far lower than what *Saina et al. (2005)* obtained 1480 g from Zimbabwe guinea fowl . The variation might be genetic or breed effect. The weight here are far lower compared to chickens of about same age, as broilers reach 2kg at 8weeks . The light weight and small body frame of guinea fowl may be a naturally selected trait meant for rapid take off (flight) and fast running as part of adaptive traits for survival in the wild *(Mareko et al., 2006)* . The result of carcass weight , breast weight and thigh weight obtain here is similar to what *Dahouda et al. (2009)* recorded on Benin guinea fowl fed with mucuna.

Table 1. Descriptive statistics of live body weight, linear traits and carcass characteristics of indigenous guinea fowl

Variable	mean±se	minimum	maximum	cv
Body weight (g)	1208±6.86	1010	1250	5.04
Body length (cm)	22.17±0.13	19.40	23.60	5.24
Wing length (cm)	19.38±0.08	18.20	21.00	3.70
Thigh length (cm)	8.94±0.07	8.00	10.00	6.54
Keel length (cm)	2.96±0.03	2.50	3.30	7.57
Chest circumf.(cm0	34.23±0.19	31.00	38.00	4.91
Carcass weight(g)	850.15±7.18	684.00	940.00	7.46
Breast weight (g)	267.23±1.69	225.00	288.00	5.58
Thigh weight(g)	72.39±0.64	59.00	78.00	7.76
Dressing percentage%	70.38			

Phenotypic correlation. Pearson' s coefficient of correlation matrix for body weight , body measurements and carcass traits of the guinea fowl are shown in Table 2. All the traits except keel length showed positive and significant correlations with body weight ($P<0.001$). However, highest correlations were recorded between carcass traits and body weight . Similar finding have been reported by *Vali et al. (2005)*, *Raji et al. (2009)*, *Alkan et al. (2010)* for different line of Japanese quails. The breast and the thigh are the area where there are higher muscles deposition in the body of the bird hence their high relationship with body weight. This indicate that selection for any of these carcass traits will lead to improvement in the other. Similarly it is an indication that any of these body dimension could serve as a predictor of body weight *(Yakubu and Ayoade, 2009)*. Apart from body weight , body length , wing length and thigh length show a high positive and significant($P<0.001$) correlation with carcass component. *Bochno et al. (1999)* obtained similar result in broilers, *Kleczek et al. (2006)* and *Wilkiewicz-Wawro and Szypulewska (1999)* in Muscovy duck. This shows that this morphometric traits are also reliable predictors of carcass composition in the guinea fowl.

Table 2. Phenotypic correlations among body weight , linear traits and carcass traits of guinea fowl

	BWT	BL	WL	TL	KL	CC	CCW	BRW
BL	.600***							
WL	.709***	.695***						
TL	.684***	.793***	.873***					
KL	-0.032	.202	.508***	.545				
CC	.747***	.769***	.934***	.923	.473***			
CCW	.906***	.680***	.894***	.772	.177	.879 ***		
BRW	.950***	.678***	.871***	.783	.160	.877***	.984***	
THW	.786***	.962***	.716***	.677	-0.05	.774***	.858***	.836***

***=P<0.001 BWT=body weight, BL=body length , WL =wing length ,TL=thigh length ,KL=keel length ,CC= chest circumference, CCW=carcass weight, BRW= breast weight and THL= thigh weight

Prediction of body weight , carcass weight and breast weight. Tables 3 , 4 and 5 presented the result of stepwise multiple regression of body weight , carcass weight and breast weight on linear body measurements. In body weight prediction , it reveals that when chest circumference alone was used it accounted for 55.5% of the total variation in body weight, inclusion of keel length in the model increase the proportion of the explained variance to 74.3%. The accuracy of the model was further improved (R^2 =80.9) when thigh length , body length and wing length were added to the equation. In predicting carcass weight, the result show that body weight alone accounted for 82% of the variation in carcass weight. The proportion of variance explained increases from 82 to 96.3% when wing length, keel length , thigh length and chest circumference were added. For breast weight prediction, body weight seems to be the major trait in determining breast weight. The result of stepwise regression analysis for predicting breast weight from live weight and linear traits show that body weight alone accounted for 90.2% of the variation in breast weight, this was progressively improved to 98.6% when wing length , thigh length and chest circumference were included. This result indicate that body weight can be predicted with a fair degree of accuracy from chest circumference , keel length and thigh length . This findings is consistent with what *Peter et al. (2006)* and *Yakubu et al. (2009)* observed in Nigeria indigenous chicken genotype, *Gueye et al. (1998)* in Senegal chicken and

Teguia et al. (2007) in muscovy duck. *Raji et al. (2009)* reported that the relationship between live body measurement for estimation of carcass component in vivo depends on the correlation between them, these observation was noticed here with higher correlation existing between body weight and carcass components(.906, .950 and .786) with carcass weight , breast weight and thigh weight respectively.

Table 3. Stepwise multiple regression of body weight on linear body measurements

Model	Explanatory variable Predictor	intercept	reg. coeff	SE	R^2	VIF
1	Chest circumference	282.763	27.034	2.759	.558	1.00
2	Chest circumference	391.381	35.521	2.373	.743	1.288
	Keel length		-134.420	17.769		1.288
3	Chest circumference	478.619	24.404	5.321	.766	6.851
	Keel length		-147.919	18.228		1.434
	Thigh length		37.265	16.074		7.563
4	Chest circumference	628.058	26.518	5.055	.796	6.851
	Keel length		-175.166	19.131		1.781
	Thigh length		63.412	17.171		9.728
	Body length		-16.546	5.126		3.424
5	Chest circumference	458.655	15.797	6.867	.809	13.518
	Keel length		-181.718	18.855		1.825
	Thigh length		62.107	16.728		9.740
	Body length		-15.929	4.999		3.436
	Wing length		28.168	12.584		8.286

Table 4. Stepwise multiple regression of carcass weight on body weight and linear body measurements

Model	Explanatory variable Predictor	intercept	reg.coeff	SE	R^2	VIF
1	Body weight	- 290.696	.944	.051	.820	1.00
2	Body weight	-706.282	.570	.039	.948	2.011
	Wing length		44.782	3.307		2.011
3	Body weight	-705.901	.451	.047	.957	3.452
	Wing length		58.497	4.597		4.649
	Keel length		-41.015	10.356		2.314
4	Body weight	-771.845	.503	.051	.960	4.392
	Wing length		62.949	4.916		5.593
	Keel length		-28.770	11.523		3.014
	Thigh length		-13.407	6.082		5.694
5	Body weight	-751.271	.476	.051	.963	4.585
	Wing length		53.657	5.959		8.849
	Keel length		-28.922	11.105		3.015
	Thigh length		-23.283	7.007		8.141
	Chest circumference		8.194	3. 187		13.920

Table 5. Stepwise multiple regression of breast weight on body weight and linear body measurements

Model	Explanatory variable	Predictor	intercept	reg.coeff	SE	R^2	VIF
1	Body weight		- 13.918	.233	.009	.902	1.00
2	Body weight		-90.592	.164	.006	.980	2.011
	Wing length			8.262	.477		2.011
3	Body weight		-101.984	.167	.005	.982	2.084
	Wing length			9.741	.697		4.682
	Thigh length			-2.320	.825		4.371
4	Body weight		-97.271	.161	.005	.986	2.270
	Wing length			7.586	.826		7.956
	Thigh length			-4.613	.938		6.837
	Chest circumference			1.895	.466		13.919

Several authors, *(Sehested 1986; Teixeira et al., 2006; Wood and Maefie, 1980; Delfa et al., 1996)* observed that multiple regression models developed to predict lean meat weight are dominated by live weight or carcass weight . In the present findings, prediction of both the carcass weight and breast weight seems to have been mainly influenced singly by the body weight 82 and 90.2%

Variance inflation factor (VIF) values for interrelationship between traits is shown along stepwise multiple regression, it represent the increase in variance due to high correlation between predictors *(Pimentel et al., 2007)*. In the present study the VIF gave indication of existence of severe collinearity (13.518, 13.520 and 13.919) in Tables 3, 4 and 5. According to *Gill (1986)* VIF greater than 10 .00 indicate severe collinearity rendering the reliability of the predictive equation not effective. It can then be suggested that the best equation for predicting body weight, carcass weight and breast weight should be

BWT=628.058+26.518CC +-175.166KL+63.412TL+-16.544BL

CCW= -771.845+.503BWT+62.949WL+-28.770KL+-13.407TL

BRW=-101.984+.167.BWT+9.741WL+-2.320TL

Conclusion

The result from this study shows that body weight , wing length and chest circumference had high positive and significant ($P<0.001$) correlation with carcass traits . Similarly body weight was shown to be a better predictor of the carcass components. This will help in providing a platform for designing breeding index for guinea fowl improvement.

In vivo predviđanje telesne mase i kvaliteta trupa korišćenjem telesnih mera kod domaće biserke

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Rezime

Ciljevi ove studije su da se proceni veza između telesne mase i kvaliteta trupa, i da se razvije linearni regresioni model za predviđanje telesne mase i niza osobine kvaliteta trupa kod domace biserke. Dvadesetosam odraslih ptica oba pola je korišćeno u istraživanju. Telesna masa i telesne mere su dobijene pre klanja, a osobine kvaliteta trupa su utvrđene na toplim trupovima. Rezultati dobijeni pomoću deskriptivne statistike su pokazali da su prosečne vrednosti osobina 1208 ± 6.86g, 22.17 ± 0.13 cm, 8,94 ± 0,07cm, 2,96 ±0.03cm, 34.23± 0.19cm, 850,15 ±7.18gr, 267,23 ± 1,69g 72,39±0,64g i 70.38% zatelesne masu, dužinu tela, dužinu buta, dužinu kobilice, obim grudi, masu trupova, masu grudi, masu buta i randman. Sve osobine izuzev dužine kobilice dužine su u pozitivnoj korelaciji sa telesnom masom (P <0,001). Osobina obim grudi je imala najveću moć predviđanja procene telesne mase (R^2 .558), dok se telesna masa izdvaja kao najvažnija promenljiva za predviđanje mase trupova i grudi (R^2 .820 i .902). Ovo nam govori da je za predviđanje mase trupova i grudi najbolje koristiti telesnu masu, ako razvijamo model za izbor i unapređenje biserki u proizvodnji mesa.

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FATTY ACID COMPOSITION OF MILK FROM TETEVEN NATIV SHEEP IN MOUNTAIN REGION

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Communication

Abstract: The study was performed on individual milk samples obtained on the monthly basis during the lactation period from 5 Teteven sheep reared in the Sredna Stara Planina mountain region. The fat extraction of milk samples was done by the Rose-Gottlieb method. Fatty acid composition was determined on a gas chromatograph with flame ionization detector and capillary column. The trends in fatty acid concentrations in sheep milk during the lactation were variable. The proportion of saturated fatty acids in Teteven sheep milk during the lactation was relatively high and varied from 70.34 to 72.19%. Variations in myristic acid concentrations by months were not significant (11.49–11.83%). The total amount of polyunsaturated fatty acids in the studied milk was relatively low and with similar values for the 4 months spent on pastures (3.78-4.11%). For this period, monounsaturated fatty acids, represented mainly by oleic acid (C18:1) decreased from 25.76 to 24.20%. The short-chain fatty acid concentrations were the highest in milk samples obtained in April and May, of medium-chain ones – in June and July, whereas long-chain fatty acids concentrations were similar over the grazing period.

Key words: sheep milk, milk fat, fatty acids

Introduction

Sheep husbandry is oriented to production of milk with high protein and milk fat content. Taking into consideration the effect of unsaturated fatty acid on human organism *(Parodi, 1999; Ryali et al., 1999; Jarheis, 2000)* the research was further focused on milk fat profile. *Signorelli et al. (2008)* did not observe any differences in the polyunsaturated fatty acid content in milk fat of native Italian breeds, including conjugated linoleic acid (CLA) concentrations. Having studied milk fat composition in Spanish Churra sheep, *Sanchez et al. (2010)* established a low inheritance with regard to saturated and monounsaturated fatty acid content and a potential for genetic variation in polyunsaturated fatty acid content.

Feeding appropriate rations could alter the fat content of sheep milk *(Addis et al., 2005)*. Changes in sward composition also influence the seasonal fatty acid content of milk fat *(Nudda et al., 2005)*. Apart fresh grass feed, sunlight exposure was reported to result in high short-chain fatty acid levels, higher lauric, myristic and stearic acid concentrations and to reduction in oleic, linoleic and linolenic acids *(Agustino et al. 2002)*.

The purpose of this investigation was to monitor the changes in milk fatty acid content during the lactation period of native Teteven sheep reared in Sredna Stara Planina region, with regard to milk fat quality evaluation.

Materials and Methods

The milk fatty acid profile in the local Teteven breed of sheep during the grazing period was studied. Five ewes at similar age and at their third month of lactation were grazed on natural pasture without concentrate. Sampling began immediately after weaning and was performed four times in a monthly interval. Samples were taken from the morning milk only and were stored at – 20 ºC. Milk fat extraction was done in the laboratory of the Dairy Science Unit at the Faculty of Agriculture, Trakia University, Stara Zagora by the method of Rose-Gottlieb. Methyl esters of fatty acids were separated on a gas chromatograph "Pay-Unicam 304" equipped with flame ionization detector and capillary column EC^{TM}-WAX, 30 m, ID 0.25 mm, Film: 0.25 μm.

Data were statistically processed by Statistica for Windows (Release, 4.3, Stat. Soft. Inc., 1994), and means were compared by Student's t-test.

Results and Discussion

Table 1 presents the results for saturated fatty acid content in the milk of Teteven sheep. Butyric acid (C4:0) concentrations decreased from April to July), while C6:0 levels increased from April to June and decreased in July. Caprilyc acid (C8:0) was high during the first two months (April and May) and decreased until July. Variations in C10:0 concentrations were not significant. Milk fat C4:0, C6:0 and C8:0 concentrations were higher than those reported for Karakachan sheep *(Mihaylova et al., 2008)* and sheep of the Srednostaroplaninska breed reared in the Sredna Stara Planina mountain *(Gerchev et al., 2011)*, but the tendency of change over the lactation was similar, probably due to the different pasture sward.

Lauric acid (C12:0) levels persisted high during the entire period and tended to decrease in July (Table 1). In May and June, only traces of C13:0 could be found. Myristic acid (C14:0) in Teteven sheep milk was almost equal during the entire period of the study. The amount was significantly higher than values reported for Karakachan and Tsigay sheep breeds *(Mihaylova et al., 2008; Gerchev*

and Mihaylova, 2009) and similar with the Srednostaroplaninska sheep breed *(Gerchev et al., 2011)*. Palmitic acid (C16:0) concentrations decreased in May and then increased until July. Among saturated fatty acids with odd number of carbon atoms, C15:0 variations were inconsistent whereas C17:0 tended to increased from April until July. Stearic acid (C18:0) levels showed the same trend of change as margaric acid (C17:0) – increase from April to July. The concentrations of C16:0, C17:0 and C18:0 in Teteven sheep milk were considerably higher than those reported in Karakachan and Tsigay sheep *(Mihaylova et al., 2008; Gerchev and Mihaylova, 2009)* and similar with the Srednostaroplaninska sheep breed *(Gerchev et al., 2011)*.

Table 1. Saturated fatty acids content

Fatty acids	April		May		June		July	
	x	Sx	x	Sx	x	Sx	x	Sx
C4:0	4.335**	0.362	3.953	0.213	3.383	0.493	2.690**	0.070
C6:0	2.476*	0.087	3.185	0.199	3.496*	0.399	2.692	0.242
C8:0	3.104	0.500	3.359	0.246	2.643	0.298	2.566	0.299
C10:0	5.994	0.408	6.160	0.600	5.302	1.241	6.090	0.247
C12:0	4.054	0.296	4.184	0.181	3.542	0.361	3.405	0.192
C13:0	-		0.186	0.087	-		0.022	0.012
C14:0	11.491	0.323	11.699	0.275	11.833	0.371	11.816	0.530
C15:0	0.562	0.104	0.846	0.196	0.545	0.057	0.751	0.081
C16:0	25.678	0.532	23.815	0.444	25.863	1.385	27.871	0.484
C17:0	0.700	0.270	1.072	0.500	1.345	0.113	1.261	0.086
C18:0	12.037	0.456	12.612	0.396	12.388	0.608	13.030	0.387

*p<0.05
**p<0.001

The content of unsaturated fatty acids – C10:1 and C12:1 in milk fat was low and changed inconsistently during the lactation months, whereas C14:1 was determined at low amounts by the beginning of lactation and in June (Table 2). The change in palmitoleic acid (C16:1) in studied samples tended to decrease slightly in May, April and then increased until the end of lactation. Milk fat oleic acid (C18:1) concentrations were gradually decreasing and reached the lowest value by lactation end. The percentage of C18:1 was the highest at the beginning of the grazing period. In many studies performed during grazing period, a positive correlation was found between C18:1 and conjugated linoleic acid (CLA) concentrations, with C18:1, particularly some isomers, are substrate for CLA synthesis.

Table 2. Unsaturated fatty acids content

Fatty acids	April		May		June		July	
	x	Sx	x	Sx	x	Sx	x	Sx
C10:1	0.273*	0.027	0.220	0.023	0.058	0.055	0.191*	0.016
C12:1	0.048	0.006	0.023	0.016	0.022	0.015	0.051	0.016
C14:1	0.018*	0.014	-	-	0.398*	0.090	-	-
C16:1	0.448	0.147	0.414	0.130	0.543	0.187	0.646	0.131
C18:1	24.973*	0.381	24.402	0.426	23.996	0.254	23.315*	0.557
C18:2	2.414	0.168	2.593	0.141	2.589	0.081	2.798	0.380
C18:3	1.369	0.112	1.408	0.090	1.257	0.108	1.312	0.108

* $p<0.05$

Polyunsaturated linoleic fatty acid (C18:2) increased during the lactation but the alterations in linolenic acid (C18:3) were various. The levels of these two acids in the milk of Karakachan, Tsigay and Srednostaroplaninska sheep breeds reared in Rhodopes and Stara planina mountains, were higher than those in Teteven sheep *(Mihaylova et al., 2006; Mihaylova et al., 2008; Gerchev et al., 2011)*.

The content of main fatty acid groups is shown n Table 3. The total amount of saturated fatty acids (SFA) during lactation was high and ranged from 70.07% in May to 72.19% in July. Monounsaturated fatty acid levels (MUFA) were higher at the beginning of lactation (April-June) and decreased in July. Polyunsaturated fatty acids (PUFA) remained with similar values over the entire lactation period. A similar ratio of the fatty acid groups in the milk of Pleven Blackhead sheep was observed by *Alexiev (2010)*. MUFA are beneficial for people with coronary and cardiovascular diseases, the effect of PUFA is similar but they are more prone to oxidation due to the higher degree of unsaturation.

Short-chain fatty acid content (Table 3) was the highest in milk produced during April and May, of medium-chain fatty acids – in June and July, and long-chain fatty acids maintained stable concentrations during the entire lactation period. The time course of short- and medium-chain fatty acids corresponded to results reported for the milk of Karakachan and Tsigay sheep reared in Rhodopes and Sredna Stara Planina mountains *(Mihaylova et al., 2006; Mihaylova et al., 2008)*, whereas the concentrations of long-chain fatty acids was lower.

Table 3. Groups of fatty acids in milk from sheep

	April		May		June		July	
Fatty acid groups	x	Sx	x	Sx	x	Sx	x	Sx
Σ SFA	70.43	3.338	71.07	3.337	70.34	4.928	72.19	2.630
Σ MUFA	25.76	0.575	25.06	0.595	25.02	0.601	24.20	0.720
Σ PUFA	3.78	0.280	4.00	0.231	3.85	0.189	4.11	0.488
Σ C4:0-C11:0	15.91	1.357	16.30	1.258	14.83	2431	14.04	0.858
Σ C12:0-C16:1	41.30	1.422	41.17	1.299	42.89	2.466	44.56	1.436
Σ C17iso-C25:0	41.49	1.164	42.09	1.553	41.57	1.164	41.72	1.518

The milk of Teteven sheep was characterized with high concentrations of saturated fatty acids and respective lower levels of MUFA and PUFA. These results correspond to what was reported by *Alexiev (2010)* in Pleven Blackhead sheep milk and the results of *Gerchev et al. (2011)* for Staroplaninska sheep milk. The omega-6/omega3 ratio, which is important for healthy diets, is low and gradually increased over the lactation – from 1.76 in April to 2.13 in July. The milk fat atherogenic index changed from 2.38 in April to 2.55 in July. The biologically important PUFA/SFA ratio (also called P/S ratio) of sheep milk is low and changed insignificantly: from 0.054 in the beginning of grazing to 0.057, proving that the changes in milk fat content over the grazing period were small and in general, maintained a balanced level. The observed ratios of fatty acids in Teteven sheep milk were considerably lower than those reported in Karakachan and Tsigay sheep breeds reared in the same region *(Mihaylova et al., 2008; Gerchev and Mihaylova, 2009)*. This could be probably attributed to the higher content of SFA and could be interpreted as a specific feature of this sheep breed.

Conclusion

The proportion of saturated fatty acids in Teteven sheep milk during the lactation was relatively high and varied from 70.34 to 72.19%. Myristic acid concentrations ranged between 11.49% and 11.83%)

The total amount of polyunsaturated fatty acids in the studied milk was relatively low and with similar values for the months spent on pasture (3.78-4.11%). Monounsaturated fatty acids, represented mainly by oleic acid (C18:1) decreased from 25.76 to 24.20%.

The short-chain fatty acid concentrations were the highest in milk obtained in April and May, of medium-chain ones – in June and July, whereas long-chain fatty acids concentrations were similar over the grazing period.

Sadržaj masnih kiselina u mleku ovce tetevenske rase

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Rezime

Istraživanja su sprovedena na individualnim uzorcima mleka dobijenih mesečnim prikupljanjem u toku laktacije 5 ovaca rase Teteven u regionu Srednje Stare planine. Ekstrakcija masti iz uzoraka mleka radjena je Rose-Gottlieb metodom. Sadržaj masnih kiselina određen je gasnim hromatografom sa plamen jonizacionim detektorom i kapilarnim kolonama.

Nivo masnih kiselina u ovčijem mleku u periodu laktacije bio je veoma varijabilan. Udeo zasićenih masnih kiselina u mleku Teteven ovce u periodu laktacije bio je relativno visok i varirao je od 70.34 do 72.19%. Varijacije u koncentraciji miristinske kiseline po mesecima nisu bile značajne (11.49–11.83%).

Ukupna količina poli-nezasićenih masnih kiselina u ispitivanom mleku bila je relativno niska i ujednačena u ispitivanom pašnom periodu (3.78-4.11%). Za ovaj period, mono-nezasićene masne kiseline, koje su predstavljene uglavnom sa oleinskom kiselinom (C18:1) su se smanjile od 25.76 do 24.20%.

Koncentracija masnih kiselina kratkog lanca bila je najveća u uzorcima mleka uzetih u aprilu i maju, srednjeg lanca u junu i julu, dok je koncentracija masnih kiselina dugog lanca bila jednaka tokom čitavog pašnog perioda.

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THE INFLUENCE OF THE TIME OF IMPLEMENTATION OF PMSG ON SOME OF THE REPRODUCTIVE PARAMETERS IN SHEEP WITH SYNCHRONIZED OESTRUS

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Communication

Abstract: Different schemes of PMSG treatment on some reproductive parameters in sheep were studied. Two groups of 6 sheep each at the end of the non breeding season were treated with vaginal sponges Chrono-gest (Intervet, Holland) containing 30 mg fluorogeston acetate (FGA). The sponges were removed 12 days after insertion. Sheep of the experimental group were injected with 500UI Folligon (Intervet, Holland) 48 hours before sponge removal whilst the corresponding control sheep were treated with the same dose Folligon at the day of of sponge withdrawal. The clinical signs of oestrus, plasma progesterone concentrations and changes in the ovarian morphology during the observation period were registered. The conception rate, fertility and twinning rate were also recorded. The results show that the earlier application of the gonadotropin influence positively the reproductive traits in ewes.

Key words: sheep, estrous synchronization, treatment

Introduction

Most of the sheep breeds reared in Bulgaria exhibit seasonal reproductive rhythmicity associated with changes in the natural dark-light cycle. Different schemes of estrous synchronization has been studied in order to improve reproductive efficiency *(Kusina et al., 2000)* adjusting the lambing time to the most favorable time of the year in relation to the forage availability, climatic conditions and market trends *(Karaca et al., 2009)*. According to *Jainudeen et al. (2000)* reproductive photoperiodicity may successfully be modified by progesterone treatment which would be expected to lengthen the luteal phase of the cycle. *Ungerfeld et al. (2002*) recommended the application of progestagens in the form of vaginal sponges for a period of 6-14 days in combination with PMSG. It was found that gonadotropins stimulate follicular growth, increase the ovulation rate

and fertility and improve synchronization *(Cline et al., 2001; Maurel et al., 2003; Ralchev et al., 2008)*.

The aim of the current study was to evaluate the effect of the various schemes of PMSG treatment on estrous synchronization, fertility and conception rate in sheep treated with progestagen, at the end of anestrus.

Materials and Methods

The study was conducted in the experimental farm of the Institute of Mountain Stockbreeding and Agriculture (Troyan) in August (late anestrus). Two groups of 6 Tsigai and Drysdale crosses each were kept indoor and daily ration consisting of 1.5 kg./head meadow hay (9.98% crude protein and 34.9% crude fiber) and 0.2 kg/head concentrate (14.3% crude protein and 7.9% crude fiber) was fed. Water and salt were offered ad libitum. Each ewe received an intravaginal sponge Chrono-gest (Intervet, Holland) impregnated with 30 mg fluorogestone acetate (FGA) for 12 days. The experimental sheep were injected with 500 UI Folligon (Intervet, Holland), 48 hour before sponge removal whereas the controls received the same treatment on the day of sponge withdrawal. The onset of estrous was detected twice daily – in the morning and in the afternoon by using a teaser ram. Ewes were checked visually and were considered to be in estrous when they allowed to be mounted. Sheep in estrous were hand-mated by a ram of the corresponding breed 24 to 72 hour after sponge withdrawal. Conception rate, fertility and twinning rate were calculated according to ewe performance at lambing.

Jugular venous blood samples were collected from three sheep of each group at the time of sponge insertion, on the day of their withdrawal and in two day intervals during the period of 16 days after mating. After centrifugation (4000 rpm for 10 min) the blood samples were stored at -20^0C up to the end of the observation when they were analyzed. In both groups of sheep uterus and ovarian were monitored and photographs were taken 72 hour after sponge withdrawal and again 17 days thereafter by means of micro camera Ricohn (Ricoh company, Japan) equipped with TTL flash Karl-Schorz-Endoskope (Germany). Laparoscopy was performed after 12 hours of food and water deprivation according to the method described by *Ralchev (1992).*

Results and Discussion

All ewes injected with Folligon 48 hours before the sponge removal showed estrous 24 – 36 hours after sponge withdrawal and some sheep continued to manifest signs of estrous during the next 12 hours. In ewes injected with PMSG on the day of sponge removal estrous occurred between 48 and 60 hours after treatment. Similar response pattern to exogenous hormone treatment in anestrous ewes was observed by *Dogan and Nur (2006)* in Kivircik breed and by *Hristova et*

al. (2010) in Il de France ewes. *Bonev et al. (2002)* also found that Il de France ewes manifested estrous 45 hours after PMGS injection. Before sponge insertion and during the first days after their withdrawal plasma progesterone concentrations were lower than 0.3 ng/ml. We also found that seventy two hours after gonadotropine injection progesterone concentration start to increase achieving in both groups the maximum values of 0.5 ± 0.16 and 0,4 ±0,13 ng/ml, respectively, by the 92nd hour after treatment and remained elevated up to the end of the observation.

Similar pattern in temporal changes of progesterone concentration was observed in Kivircik ewes treated with MAP (medroxiprogesteron acetat), 500 UI PMSG and 5 mg Dinaprost at the time of sponge removal *(Ekiz and Ozcan, 2006)*. Progesterone concentration started to increase 50 h after injection and at 122 h it already exceeded 0.5 ng/ml. Our results corresponded closely to the findings of *Ralchev et al. (2008)* who studied the changes in plasma progesterone concentrations in Tsigai ewes injected with 500 and 1000 UI gonadotropin at the time of sponge removal during the non-breeding season. In both groups of sheep progesterone concentration began to increase 72 h after gonadotropin injection reaching the peak levels of 0.47 and 0.33 ng/ml, respectively, by the 5th day post-treatment. The low level of plasma progesterone at the beginning of the estrous found in our study may be accounted for by the functional changes related to development and maturation of corpus luteum. According to *Barret et al. (2002)* progesterone levels lower than 0.2 ng/ml may be considered as a baseline.

The number of corpus luteum monitored at 72 h after sponge removal was equal in both groups whilst on day 17 it was higher in ewes injected with PMGS 48 h before sponge withdrawal (Table 1).

Table 1. Data from the laparoscopy performed at 72 h and 17 days after removal of the vaginal sponges

Sheep №	72nd hour		17th day		Lambs born
	right ovary	left ovary	right ovary	left ovary	
Experimental group					
170	non-observed	corpus rubrum (12 hours)	normal	gravid corpus luteum	2
701	3 corpora lutea	normal	2 gravid corpora lutea	normal	3
702	normal	corpus rubrum	normal	1 gravid corpus luteum	barren
704	normal	1 corpus luteum	normal	1 corpus luteum	1
705	1 corpus luteum	non-observed	1 corpus luteum	follicles	1
706	normal	corpus rubrum	normal	corpus rubrum	barren
Control group					
707	cyst	1 corpus luteum	normal	1 corpus luteum	1
709	normal	corpus rubrum	normal	corpus albicans	barren
710	1 corpus luteum, 3-4 cystic changes	1 non-ovulated follicle 1 corpus rubrum	1 corpus luteum	normal	2
715	3 cysts	1 corpus luteum	normal	1 corpus luteum	barren
718	normal	cyst	normal	corpus albicans	barren
719	1 corpus luteum	3 cysts	1 corpus luteum	normal	1

The incidence of ovarian cysts tended to be higher in sheep treated with PMGS at the time of sponge removal. This, in turn, may affect plasma progesterone concentration and influence negatively conception rate and fecundity.

Generally, our results suggest that time of PMSG treatment affect reproductive traits. Conception rate, fertility and twinning rate were 66.7%, 116.6% and 50% in experimental ewes and 50.0%, 66.6% and 33.3%, respectively, in control sheep (Figure 1).

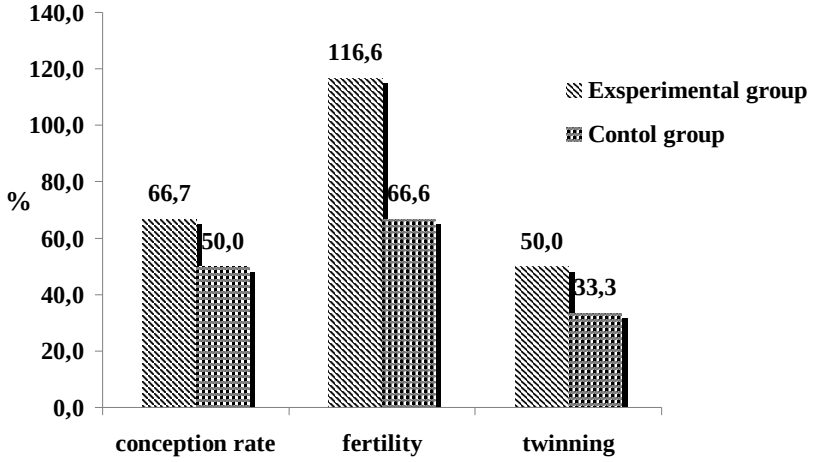

Figure 1. Reproductive traits in experimental and control ewes

Koyuncu and Ozis (2010) reported the highest values of in Kıvırcık ewes treated over the breeding season. These authors pointed out that in ewes injected with PMSG 24 h before sponge removal conception rate, fertility and twinning rate constituted 76.7%, 132.0% и 73.9%. The corresponding values in sheep receiving the same treatment at the time of sponge withdrawal were 86.2%, 127.6% and 61.9%, respectively. Similarly, *Zeleke et al. (2005)* found that PMSG treatment 24 h before sponge withdrawal increased fertility in sheep. The overview of findings showed that responses to hormonal treatment may vary according to breed, season, treatment regimes, management and mating system.

Conclusion

The data derived from the current study suggest that application of PMSG before sponge removal affect positively reproductive performance of sheep.

Folligon treatment at the time of sponge removal cause ovarian abnormalities increasing the incidences of ovarian cysts.

Uticaj vremena implementacije PMSG na reproduktivne parametre ovaca sa sinhronizovanim estrusom

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Rezime

U radu su prikazana ispitivanja uticaja različitih šema PMSG tretmana na reproduktivne parametre kod ovaca. Dve grupe od po 6 ovaca su na kraju vansezonskog perioda oplodnje tretirane vaginalnim sunđerima natopljenim Chrono-gest-om (Intervet, Holland) koji sadrži 30 mg fluorogeston acetata (FGA). Sunđeri su uklonjeni 12. dana nakon aplikacije. Ovce eksperimentalne grupe su tretirane sa 500UI Folligon (Intervet, Holland) 48 sati pre vađenja sunđera dok su ovce kontrolne grupe tretirane sa istom dozom Folligena na dan vađenja sunđera. Praćeni su klinički znaci estrusa, koncentracija plazma progesterona, promene u morfologiji ovarijuma, koncepcija, plodnost i nivo bliznjenja. Rezultati pokazuju da ranija primena gonadotropina utiče pozitivno na reproduktivne sposobnosti ovaca.

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THE APPLICATION OF DIFFERENT METHODS FOR THE DETERMINATION OF FAT CONTENT IN SELECTED ANIMAL – ORIGIN PRODUCTS

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Abstract

Abstract: The aim of the study was to assess the fat content in selected animal-origin products (ie. black pudding, luncheon, wiener, bacon) as well as certified reference material (MUVA RM700 Boiled Sausage) using two analytical methods. One of them was the fat extraction using a Soxhlet technique, and the other one – the extraction using carbon dioxide in supercritical state. Methods based on Soxhlet extraction have been and are at present used as reference. This conventional method is cheap and accurate, and this is why it has been the most used extraction technique worldwide for a number of decades. On the other hand some modern methods of fat determination are needed. Supercritical Fluid Extraction is quick, safe, and it eliminates any hazardous chemicals or solvents used in traditional extraction methods. For this purpose we used TFE 2000 fat analyzer (LECO Corporation). For the methods used the following validation parameters were determined: accuracy, precision, intermediate precision, correctness. The fat content in black pudding and luncheon meat ranged from 11.5 ± 4.5 g/100g (TFE) to 12.1 ± 0.0 g/100g (Soxhlet) and from 13.2 ± 0.5 g/100g (Soxhlet) to 13.8 ± 0.7 g/100g (TFE), respectively. For wiener samples we received values between 15.7 ± 0.4 g/100g (TFE) to 17.8 ± 0.1 g/100g (Soxhlet). The fat content in bacon samples ranged from 48.2 ± 2.5 g/100g (TFE) to 48.7 ± 0.7 g/100g (Soxhlet). The results received remain consistent with the values given in the Polish "Food Composition Tables". The application of the methods described above for the determination of fat content in animal – derived products is dependent ie.: on the purpose of analysis, the amount of fat in food product sample and the time for receiving the results.

Primena različitih metoda za određivanje sadržaja masti u odabranim proizvodima životinjskog porekla

M. Surma-Zadora, K. Topolska, E. Cieślik, K. Sieja

Rezime

Cilj ovog istraživanja je bio da se oceni sadržaj masti u odabranim proizvodima životinjskog porekla (npr. crni puding, meso, viršla, slanina) kao i sertifikovani referentni materijal (MUVA RM700 Boiled Sausage) korišćenjem dve analitičke metode. Jedna je ekstrakcija masti korišćenjem metode po Soxhlet-u, a druga – ekstrakcija korišćenjem ugljendioksida u superkritičnom stanju.

Metode koje se zasnivaju na ekstrakciji po Soxhlet-u se još uvek koriste kao referentne metode. Ova konvencionalna metoda je jeftina i tačna, i zbog toga predstavlja tehniku ekstrakcije koja se najviše koristi u svetu. S druge strane, neke moderne metode za određivanje sadržaja masti su neophodne. Superkritična tečna ekstrakcija predstavlja brzu, bezbednu metodu koja eliminiše sve opasne hemikalije ili rastvarače koji se koriste u tradicionalnim metodama ekstrakcije. U ovu svrhu koristili smo TFE 2000 analizator masti (LECO Corporation). Za metode korišćene, određivani su sledeći parametri validacije: tačnost, preciznost, srednja preciznost, ispravnost.

Sadržaj masti u crnom pudingu i mesu bio je od 11,5 ± 4,5 g/100g (TFE) do 12,1 ± 0,0 g/100g (Soxhlet) i od 13,2 ± 0,5 g/100g (Soxhlet) do 13,8 ± 0,7 g/100g (TFE), respecktivno. U uzorcima viršle dobijeni su sledeći rezultati 15,7 ± 0,4 g/100g (TFE) do 17,8 ± 0,1 g/100g (Soxhlet). Sadržaj masti u uzrocima slanine bio je od 48,2 ± 2,5 g/100g (TFE) do 48,7 ± 0,7 g/100g (Soxhlet). Dobijeni rezultati su ostali dosledni u okviru vrednoti navedenih u poljskim "Tablocama sastava prehrambenih naimirnica".

Primena metoda opisanih gore za određivanje sadržaja masti u proizvodima životinjskog porekla zavisi od: svrhe analize, količine masti u uzorku prehrambenog proizvoda i vremena dobijanja rezultata.

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THE CONTENT OF NUTRIENTS, PRESERVATIVES AND CONTAMINANTS IN SELECTED ANIMAL-ORIGIN PRODUCTS FROM DIFFERENT MANUFACTURERS

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Abstract

Abstract: The aim of this study was to compare the quality of animal – origin products from different leading manufacturers. The research material were kabanos, black pudding, sausage, luncheon meat, homogenized sausage, liverwurst, headcheese, ham, bacon and minced meat. A dry matter, nutrient content, preserwatives (nitrates, phosphates and salt) and contaminants (heavy metals and pesticides) were determined in all samples. The total phosphorus content (expressed as P) was determined using vanadomolybdate method (UV-Vis spectrophotometry). The determination of salt was conducted using Mohr titration method. For nitrate and nitrites amount determination enzymatic method was used. The fat content was determined using Soxhlet method extraction. Truspec was used for proteins content determination. Heavy metal and minerals were determined using AAS. The total phosphorus content in meat products ranged from 82 mg/100g (liverwurst) to 313 mg/100g (ham). The sodium chloride content in the samples ranged from 0.39% (minced meat) to 2.67% (kabanos). For nitrates and nitrites the results ranged from 3.11 mg/kg (kabanos) to 69.90 mg/kg (bacon) nitrates and from 3.52 mg/kg (black pudding) to 78.71 mg/kg (homogenized sausage) nitrites. The obtained results for fat content were ranged from 48.7 g/100g (bacon) to 3.5 g/100g (ham). The results for proteins content ranged from 7.9 g/100g (liverwurst) to 26.6 g/100g (kabanos). The results ranged from 53.88 mg/kg (liverwurst) to 289.61 mg/kg (luncheon meat) for calcium, from 40.41 mg/kg (liverwurst) to 274.96 mg/kg (kabanos) for magnesium, from 0.65 g/kg (minced meat) to 4.70 g/kg (kabanos) for potasium, from 0.65 g/kg (minced meat) to 15.2 g/kg (kabanos) for sodium, from 0.12 mg/kg (luncheon meat) to 1.15 mg/kg (liverwurst) for copper, from 5.38 mg/kg (backon) to 58.54 mg/kg (black pudding) for iron, from 0.08 mg/kg (backon) to 2.44 mg/kg (black pudding) for manganese, from 8.35 mg/kg (homogenized sausage) to 33.24 mg/kg (minced meat) for zinc and from 0.001 mg/kg (homogenized ham) to 0.011 mg/kg (black pudding) for cadmium.

Sadržaj hranljivih materija, konzervanasa i kontaminanata u odabranim životinjama – poreklo proizvoda od različitih proizvođača

M. Surma-Zadora, A. Sadowska-Rociek, E. Cieślik, M. Walczycka, K. Sieja, L. Wałkowska

Rezime

Cilj ove studije bio je da se uporedi kvalitet proizvoda životinjskog porekla od vodećih proizvođača. Predmet istraživanja su bili sledeći proizvodi – kabanos, crni puding, kobasica, homogenizovana kobasica, jetrena pašteta, šunka, slanina i mleveno meso. Suva materija, sadržaj hranljivih materija, konzervanasa (nitrati, fosfati i so) i kontaminanti (teški metali i pesticidi) su određivani u uzorcima.

Ukupni sadržaj fosfora izražen kao P, određivan je korišćenjem vanadomolibdat metode (UV-Vis spektro fotometrija). Sadržaj soli je određivan metodom Mohr titracije. Količina nitrita i nitrata je određivana enzimskom metodom. Sadržaj masti je određivan metodom ekstrakcije po Soxhlet-u. Truspec je korišćen za određivanje sadržaja proteina. Teški metali i minerali su određivani korišćenjem AAS.

Ukupni sadržaj fosfora u proizvodima od mesa bio je od 82 mg/100g (pašteta) do 313 mg/100g (šunka). Sadržaj natrijum hlorida u uzoricma bio je od 0,39% (mleveno meso) do 2,67% (kabanos). Za nitrate i nitrite rezultati su bili od 3,11 mg/kg (kabanos) do 69,90 mg/kg (slanina) nitrata, i od 3,52 mg/kg (crni puding) do 78,71 mg/kg (homogenizovana kobasica) nitrita. Dobijeni rezultati za sadržaj masti bili su od 48,7 g/100g (slanina) do 3,.5 g/100g (šunka). Rezultati sadržaja protein bili su u opsegu od 7,9 g/100g (pašteta) do 26,6 g/100g (kabanos). Rezultati su bili u opsegu od 53,88 mg/kg (pašteta) do 289,61 mg/kg (meso) za kalcijum, od 40,41 mg/kg (pašteta) do 274,96 mg/kg (kabanos) za magnezijum, od 0,65 g/kg (mleveno meso) do 4,70 g/kg (kabanos) za kalijum, od 0,65 g/kg (mleveno meso) do 15,2 g/kg (kabanos) za natrijum, od 0,12 mg/kg (meso) do 1,15 mg/kg (pašteta) za bakar, od 5,38 mg/kg (slanina) do 58,54 mg/kg (crni puding) za gvožđe, od 0,08 mg/kg (slanina) do 2,44 mg/kg (crni puding) za mangan, od 8,35 mg/kg (homogenizovana kobasica) do 33,24 mg/kg (mleveno meso) za cink i od 0,001 mg/kg (homogenizovana šunka) do 0,011 mg/kg (crni puding) za kadmijum.

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THE CONTENT OF FAT AND FATTY ACIDS COMPOSITION IN CHICKEN LIVER

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Abstract

Abstract: Liver is recognized as a valuable source of nutrients in human nutrition but there are a few reports of the lipid profile of chicken liver. The aim of work was to determine fat content as well as fatty acids composition of raw chicken liver. The study was carried out on three chicken breeds (New Hampshire, Plymouth Rock, Rhode Island) obtained from National Research Institute of Animal Production in Balice. Total fat was determined by Soxhlet method and the fatty acids profile was performed with gas chromatography method on a Varian 3400. Fat content of chicken liver ranged from 2.65 to 10.07 g/100g depended on the breed. The predominant saturated acid (SFA) in all samples was palmitate (C16:0), followed by stearate (C18:0). Oleate (C18:1) was the major monounsaturated fatty acid (MUFA) in all samples, followed by palmitooleate (16:1). n-3 fatty acids in chicken liver were comparatively lower than SFA and MUFA. The predominant polyunsaturated acid (PUFA) was linoleate (C18:2). Arachidonate (C20:4) was the second most important *n*-6 fatty acid. Fatty acid content also varied between breeds of chicken. It has been demonstrated that conjugated linoleic acids (CLA) is found in chicken liver.

Key words: chicken liver, fat, fatty acids

Sadržaj masti i sastav masnih kiselina u pilećoj jetri

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Rezime

Jetra se smatra vrednim izvorom hranljivih materija u ljudskoj ishrani, ali ima vrlo malo studija koje se bave profilom lipida u jetri pilića. Cilj ovog rada je bio da se odredi sadržaj masti kao i sastav masnih kiselina sirove pileće jetre. Ispitivanje je urađeno na pilićima tri rase (novi hempšir, plimut rok, rod ajland) u nacionalnom istraživačkom institutu za stočarstvo u Balicama. Ukupni sadržaj masti je određivan metodom po Soxhlet-u a profil masnih kiselina gasnom hromatografijom na aparatu Varian 3400.

Sadržaj masti u pilećoj jetri bio je od 2,65 do 10,07 g/100g zavisno od rase. Preovlađujuća zasićena kiselina (SFA) u svim uzorcima je bila palmitinska kiselina (C16:0), zatim stearinska kiselina (C18:0). Oleinska kiselina (C18:1) je bila glavna mono-nezasićena masna kiselina (MUFA) u svim uzorcima, zatim palmitooleinska kiselina (16:1). Sadržaj n-3 masnih kiselina u pilećoj jetri je bio relativno nizak u odnosu na SFA i MUFA. Preovlađujuća poli-nezasićena masna kiselina (PUFA) bila je linoleinska (C18:2). Arahidonska kiselina (C20:4) je bila druga najvažnija *n*-6 masna kiselina. Sadržaj masnih kiselina je varirao zavisno od rasa. Takođe su utvrđene konjugovane linolne kisleine (CLA) u pilećoj jetri.

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COMPARISON OF CONTENT OF SELECTED MINERALS AND CADMIUM IN CHICKEN AND GOOSE LIVER

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Abstract

Abstract: The aim of the study was to compare the content of selected metals in raw chicken and goose liver. The study was carried out on three chicken breeds (New Hampshire, Plymouth Rock, Rhode Island) obtained from National Research Institute of Animal Production in Balice and Zatory Landrace goose breed obtained from Department of Poultry, Fur Animal Breeding and Animal Hygiene-Experimental Station in Rzaska. The analysis of Fe, Cu, Mn, Zn and Cd were performed in 24 samples by atomic absorption spectrometry (using Varian AA240Z and AA240FS spectrometer) after microwave digestion (MARS Xpress, CEM). The concentrations of investigated minerals in chicken liver samples were found to be in the range of 63.09-146.47 mg/kg for iron, 3.46-5.34 mg/kg for copper, 2.99-4.99 mg/kg for manganese, 40.19 and 59.42 mg/kg for zinc and 0.03-0.06 mg/kg for cadmium. The minerals content of goose liver was found to be much higher as compared to chicken liver. Minerals content in goose liver samples were to be in the range of 52.93-659.84 mg/kg for iron, 39.32-64.45 mg/kg for copper, 3.43-5.32 mg/kg for manganese, 50.87-67.20 mg/kg for zinc and 1.10-4.12 mg/kg for cadmium. The concentration of cadmium found in chicken liver in most instances is lower than the limits established by European Union legislation, in contrast to goose liver, where the concentration of cadmium highly exceeds the limits.

Key words: liver, chicken, goose, minerals, cadmium

Poređenje sadržaja odabranih minerala i kadmijuma u pilećoj i guščjoj jetri

E. Cieślik, I. Walkowska, J. M. Molina-Ruiz, I. Cieślik, W. Migdal

Rezime

Cilj ove studije bio je poređenje sadržaja odabranih metala u sirovoj pilećoj i guščjoj jetri. Istraživanje je sprovedeno na tri rase pilića (novi hempšir, plimut rok, rod ajland) Nacionalnog Instituta za stočarstvo u u Balicama i Zatory rase gusaka iz Odeljenja za uzgoj živine i krznašica i higijenu životinja-Eksperimentalna stanica u Rzaska. Analiza Fe, Cu, Mn, Zn i Cd je sprovedena u 24 uzorka atomskom apsorpcijskom spektrometrijom (koristeći Varian AA240Z i AA240F spektrometar) nakon mikrotalasne digestije (MARS Xpress, CEM).

Koncentracije ispitivanih minerala u pilećoj jetri utvrđene su u rasponu od 63,09 do 146,47 mg/kg za gvožđe, 3,46-5,34 mg/kg za bakar, 2,99-4,99 mg/kg za mangan, 40,19 i 59,42 mg/kg za cink i 0,03-0,06 mg/ kg za kadmijum. Utvrđen je sadržaj minerala u guščjoj jetri mnogo veći u odnosu na pileću jetru. Sadržaj minerala u uzorcima guščje jetre bio je u rasponu od 52,93 do 659,84 mg/kg za gvožđe, 39,32-64,45 mg/kg za bakar, 3,43-5,32 mg/kg za mangan, 50,87-67,20 mg/kg za cink i 1,10-4,12 mg/kg za kadmijum. Koncentracija kadmijuma utvrđena u pilećoj jetri u većini slučajeva je niža od granica utvrđenih zakonodavstvom Evropske unije, za razliku od guščje jetre, gde je koncentracija kadmijuma bila znatno iznad granice.

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Instruction for authors

Papers for publishing in the *Biotechnology in Animal Husbandry* journal should be submitted to the Editorial Board. Address: Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, P.O.box 23, Republic of Serbia (for *Biotechnology in Animal Husbandry*).

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Example 1

TABLE EGGS OF KNOWN ORIGIN AND GUARANTEED QUALITY - BRAND EGG

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Z. Pavlovski, Z. Škrbić, M. Lukić

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Corresponding author: zlaticapav@yahoo.com
Invited paper

Example 2

THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

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Corresponding author: petrovicm@tfc.kg.ac.yu
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Original scientific paper should contain following paragraphs with single spacing (title of paragraphs should be in Times New Roman 14 **bold,** except for **Abstract** and **Key words** where font size is 11 **bold**):

Abstract: 250 words

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Introduction - present the review of previous research and objective of the paper.

Materials and Methods - state methods applied in the paper.

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Text and titles of tables, figures and graphs, Times New Roman, font size 9, **bold**, in the following form:

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Tables and figures should be numbered and with adequate title and legend, width and height not exceeding 12 cm and 17 cm, respectively. Tables should be prepared according to instruction for forming of tables in Office Word. Each column in table must have heading and, when necessary, abbreviations should be explained in the legend/footnote.

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Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 6885.

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Z. Pavlovski, Z. Škrbić, M. Lukić

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PAVLOVSKI Z. (2004): Novi propisi EU, dobrobit živine, zahtevi potrošača. Živinarstvo, 8-9, 49-58.

PAVLOVSKI Z., MAŠIĆ B. (1994): Odnos potrošača prema živinskim proizvodima. Živinarstvo, 7-9, 77-82.

PETROVIĆ D.M., GUTIĆ M., BOGOSAVLJEVIĆ-BOŠKOVIĆ S. (2004): Masa teladi pri rođenju i njena varijabilnost kod krava simentalske rase. Agroznanje, 5, 1, 111-116.

Citations in the text are presented in *italic* form, examples: ...results of *Pavlovski (2004)...*; *(Pavlovski and Mašić, 1994)*; *(Petrović et al., 2004)*; *(Pavlovski, 2004; Pavlovski and Mašić, 1994; Petrović et al., 2004)*.

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